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DIAGNOSTIC STAGES OF URODELE METAMORPHOSIS[1]

WITH REFERENCES TO AMBLYSTOMA PUNCTATUM AND TRITURUS VIRIDESCENS

MADELEINE P. GRANT
Mount Holyoke College

NINE TEXT FIGURES AND THREE PLATES (THIRTEEN FIGURES)

The process of metamorphosis in the Urodela shows great variations in different species. The factor which varies most widely is the rate at which the changes occur. Amblystoma opacum, for example, has been observed to transform within twenty-four hours. The increase in the metabolic rate and the consequent increased demand for oxygen occur so suddenly in this species that the animal must be removed at once from an aquatic to a moist environment in order to prevent the death of the individual. The laboratory worker has, however, much more warning of the approach of metamorphic changes in Amblystoma punctatum, which requires four or five days for its completion. Triturus viridescens shows a still more retarded series of transformations which may cover a period of a week or ten days.

The external changes in all of these species involve the well-known pictures of alteration in pigment pattern, the disappearance of dorsal and ventral tail fins with simultaneous rounding of the tail, gill atrophy, bulging of the eyes, and epidermal changes which are first seen by skin sloughing. Because the study of amphibian material at the metamorphic period is attracting an ever-increasing number of investigators, it seems important to obtain detailed descriptions which will aid in the recognition of these changes as they

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[1] This study, aided by a Whitney Fellowship from Radcliffe College, was conducted at the Agassiz Museum of Comparative Zoölogy.

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occur in the common species used for experimental work. The object of the present report, therefore, is to describe 'at metamorphosis,' a term repeatedly used by authors without definition.

Growth and differentiation constitute a continuous process which only artificially can be separated into different stages. In studying the transformations which accompany the emergence of an animal from the aquatic to the moist environment, the investigator makes a false assumption when he believes that he can put his finger upon the actual initial or final step in a process so dynamic in nature as that of metamorphosis. Nevertheless, it is of utmost importance to recognize the first hint of the beginning of these changes. Study of material during a period just preceding the rapid atrophy of larval structures must be made in order to complete many of the studies dealing with amphibian metamorphosis.

Urodeles which have been experimentally forced to undergo precocious transformation by the injection of thyroxin or by feeding thyroid extract do not appear to respond at once, and for some time after treatment give no observable hint of the approaching deep-seated changes which seem to appear so suddenly. This latent period lasts from three or four days to a month, depending upon the strength and frequency of the dosage and the susceptibility of the animals used. Some delay in the atrophy of larval structures occurs in all experimental animals, even though the individuals are receiving increased amounts of the hormone considered responsible for the actual metamorphic changes. It is not improbable that the normally developing urodele passes through a period which corresponds to this latent interval in experimental animals. If such is the case, its nature cannot be determined until the criteria which foreshadow gross metamorphic changes are known. Cytological studies of the condition of the thyroid may then be associated with them.

Uhlenhuth and others have experimentally demonstrated that secretions from the anterior lobe of the pituitary gland contain elements which stimulate thyroid activity. It may be assumed that such a factor plays a part in the release of colloid in the thyroid of the normally developing animal. A study of the histogenesis of the pituitary through the metamorphic period, such as has not yet been made, may support these experimental findings and extend our knowledge of its microscopic structure.

The writer is engaged upon a series of cytological studies involving mitochondrial and Golgi techniques upon the thyroid and pituitary glands of Amblystoma punctatum and Triturus viridescens. As a preliminary step it appears to her necessary: 1) to determine the nature of a possible incipient metamorphic stage, that is, a stage preparatory to the actual reduction of larval structures, and, 2) to establish the sequence of events in the familiar metamorphic pictures and to relate their occurrence to a time schedule. The present report of the metamorphic process in Amblystoma punctatum and Triturus viridescens describes incipient metamorphic stages and the sequence of events in the atrophy of larval structures.

THE LITERATURE ON URODELE METAMORPHOSIS

Descriptions of urodele metamorphosis are found in the literature on ecology, taxonomy, anatomy, and experimental zoölogy and include reports from the following authors: Banta and McAtee ('06), Wilder ('25), Opacki ('26), Uhlenhuth ('17, '25, '27), Pope ('24), and Zawadowsky ('28). None of these investigators have established the sequence of events of the metamorphic period. Furthermore, there has been no cytological study of the thyroid and pituitary glands during premetamorphic stages.

Banta described the structural and pigment changes in the larval and adult Spelerpes maculicaudus. A series of eight photographs shows successive transformations in pigment pattern from the uniformly gray-colored larva to the adult with a flaming orange background and discrete black spots. In the fifth of these illustrations the absorption of the tail fin is discernible. The color changes were described and interpreted merely as belonging to different age periods of the animal, with no attempt to determine a pigment phase which might be used as a premetamorphic criterion. No histological studies were made during these phases.

An elaborate study of amphibian metamorphosis was made by Wilder ('25) on Eurycea bislineatus. Attention should be called to her success in establishing four different phases in the development of this species. The last two she describes as follows:

> Premetamorphic stage; Open naso-lacrimal groove (incipient phase), *os thyreoideum,* no vesicular glands in skin.
>
> Metamorphic stage; Acinous glands of the skin as tiny vesicles (incipient phase), becoming larger and more conspicuous; absorption of larval structures and development of naso-labial grooves and eyelids (advanced phase).

These histological evidences of approaching metamorphosis suggested criteria used in the present investigation. Wilder made no study upon the pituitary, and her report of the thyroid was confined to a few statements in which it is clear that the only evidence of increased thyroid activity noted was seen in the collapsed follicles which suddenly increased their output of colloid into the sinusoids in the late or advanced metamorphic period, some time during which tail and gill atrophy was taking place.

Opacki ('26) established the disappearance of the Leydig cell of the integument as a criterion of metamorphosis. This proved of value in his study of histological changes in grafted skin. As will be shown in the present report, the disappearance of this specialized integument cell of larval urodeles marks an advanced transformation stage, and therefore its use as a metamorphic criterion should be thus limited.

Recognition must here be made of the able studies on the morphology and physiology of the urodele thyroid made by Uhlenhuth during the last twelve years—work which has furnished a background for the present investigation. In 1917, he described pigment changes in Amblystoma punctatum which occur prior to tail and gill atrophy. The color changes were used to interpret his experimental results upon skin and eye transplants. There was no attempt in this nor in subsequent reports of his to study the cytology of the thyroid during these pigment changes. None of the initial color changes were used as premetamorphic criteria. No definite sequence of events was established concerning gill and tail reduction and skin molting. His later work has been concerned with the histogenesis of the urodele thyroid and with an experimental analysis of the rôle of the pituitary without a cytological study of the latter. He described the larval thyroid gland as the retention gland ("Stadium des 'schlau-artigen Follikels' "), the metamorphic gland as the colloid-releasing gland ("Stadium der 'Kolloidentleerung' "), and noted refilled follicles ("Stadium der Wiederfuellung der Follikeln") in the adult gland. He used initial skin shedding as the criterion of the metamorphic process and dated all his studies upon the metamorphic thyroid from this event. The evidence given in the present report points to skin shedding as a final, not an early event, in the metamorphic process of Amblystoma punctatum and Triturus viridescens.

Skin molting in urodeles has received a surprising amount of study. From observations upon Eurycea bislineatus, Wilder gives convincing evidence to show that, contrary to general opinion, skin molts appear in larval as well as metamorphosing individuals. The record of her experience in this observation is of interest:

> I first observed it (the flaky molt) only in individuals in the incipient phase of premetamorphosis and for a long time I considered it one of the premetamorphic phenomena until its unmistakable appearance in younger individuals forced itself upon my somewhat unwilling attention.

As early as 1857, Kneeland reported that he had observed Necturus to shed its skin in the winter. Dawson ('20) showed that the time of the year does not determine skin sloughing in Necturus and reported that this species may be made to cast its skin whenever the animal is taken from the water for a short time and allowed to dry. The present writer has found from microscopic examination of pieces of débris from the aquaria in which one- and two-year-old Necturus larvae were kept that these were often epidermal castings. It seems evident that our old concept of skin molting in urodeles as pertaining only to metamorphic individuals is open to reinvestigation.

Although Wilder established no time relationship between gill and tail atrophy and skin shedding, but stated that all these processes occurred simultaneously during the late metamorphic stage, her description of molting in the metamorphic Eurycea is significant and should be cited here:

> From the recorded observations of the appearance and molting activities of some 30 individuals during the final process of metamorphosis a certain uniformity of the sequence of events was recognized. . . .
>
> 1. Upon the approach of the final transformation process there is a period of a few days during which a copious flaky molt is cast off similar to that which takes place during larval life. . . .
>
> 2. Immediately following the flaky molt there occurs a molting process which consists of the casting off of thin torn fragments of various sizes.
>
> 3. Within a few days subsequent to this fragmentary molt, there occurs a casting off of a complete molt of the adult type, usually in two or more large pieces.

These observations, which she supported by histological descriptions of these types of molt, make it obvious that unless special attention is given to this particular problem, data pertaining to skin molting are not a precise time criterion.

The initial skin shedding here reported refers to the 'adult molt' (fig. 1). This was sometimes difficult to determine, principally because of the variations in its appearance. These variations were undoubtedly due to the presence of the 'fragmentary molt.' In the majority of cases, however, this was not seen separately, because, as suggested by Wilder, it may be cast off along with the 'adult molt.' In spite of the possibility of confusion upon the question of type of molt, the significance attached to the phenomenon in this report still holds, for it opens the possibility that the occurrence of skin shedding may even be a few days later than the time recorded. Uhlenhuth's use of initial skin shedding as the mark of the beginning of the metamorphic period has already been stated. His index is probably the 'adult molt' described by Wilder, and indicates a period when the animal is well into the metamorphic process. The following description of transformation changes in Amblystoma punctatum shows that the process started in the majority of cases at least eight or nine days prior to skin sloughing.

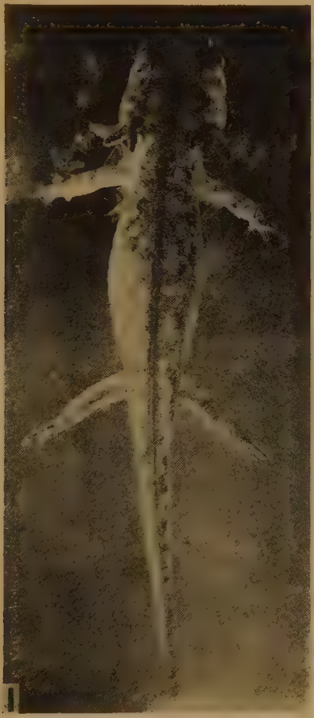


Fig. 1 Amblystoma punctatum. Initial skin shedding of typical 'adult molt.'

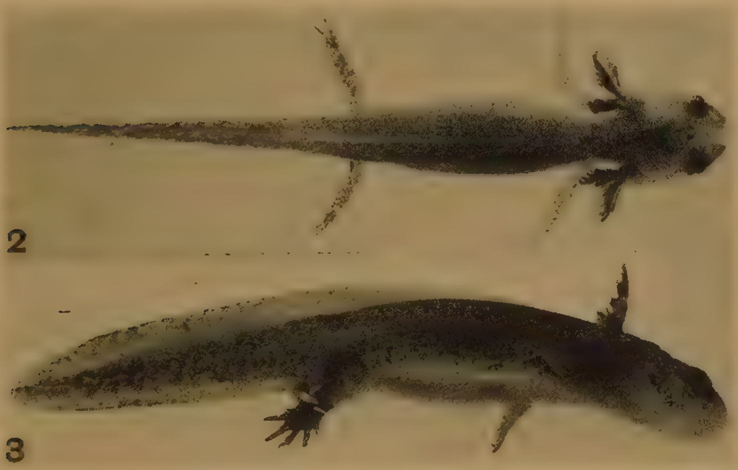
This survey of the literature dealing with urodele metamorphosis shows no attempt on the part of any investigator to establish a sequence of events of the generally accepted metamorphic changes seen in the atrophy of larval structures, and no cytological study of the pituitary gland during the transformation period. Except for the studies of Wilder, no attempt has been made to establish 'premetamorphic' and 'incipient metamorphic' criteria. Since Wilder's studies were confined to Eurycea bislineatus, a urodele freak living a year or more as a true larval individual, no general conclusions concerning the morphogenesis of the metamorphic, tailed amphibians can be made from her report. The study of thyroid histogenesis during these initial stages did not receive her attention. Furthermore, our knowledge of the histogenesis of the metamorphic urodele thyroid is confined to studies dating from initial skin molting—a phenomenon which observations here reported show to be a final event in the metamorphic picture.

MATERIAL AND METHODS

In order to establish different metamorphic stages, the investigator has two methods at his disposal: 1) daily records of changes in external structure, 2) daily determinations of the metabolic rate of the animal—a procedure obscured by experimental difficulties. In the present report the former method has been used for the study of some fifty Amblystoma punctatum and approximately as many Triturus viridescens. The studies of individuals of each species were extended by series of photographs taken at significant intervals during the growth of the same animal. This method served as a valuable record, and especially aided in establishing the sequence of events of the metamorphic process. The photographs of Amblystoma punctatum included in this report were all taken of animals raised in the laboratory from the blastula stage and fed rich diets of Daphnia and Enchytraeus and kept under identical room-temperature conditions. Several of the photographs were from animals from the same nest. The illustrations of Triturus were all taken from animals found in the field in an advanced larval condition during the middle or late summer and kept in the laboratory for three or four weeks when the metamorphic process began. All animals were photographed while under a chloretone anaesthetic.

AMBLYSTOMA PUNCTATUM

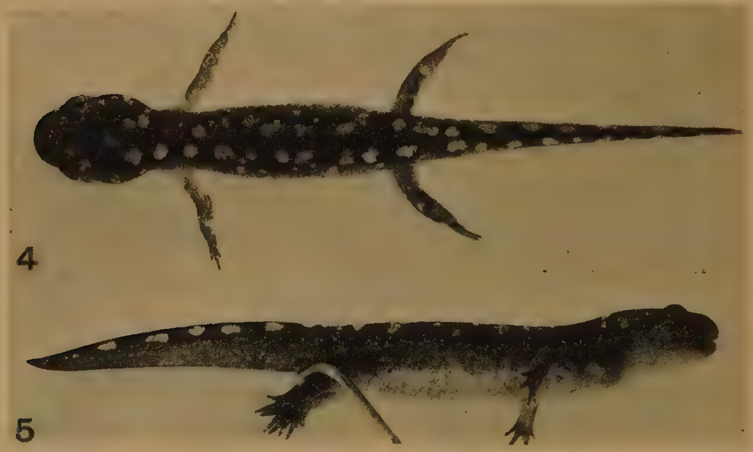
Approaching metamorphosis in Amblystoma punctatum is first indicated by the appearance of numerous small, dark areas on the homogeneous yellow-brown larva (figs. 2, 3 and pls. 1, 2). Histological studies of these color changes are necessary before any statements can be made concerning the precise nature of these pigment migrations, but a study of the photographic series on plates 1 and 2 will show that the pigment pattern which was first seen as an accumulation of numerous dark areas was maintained and extended until the adult pattern with the black background containing discrete yellow spots was reached (figs. 3 and 4).



Figs. 2 and 3 Amblystoma punctatum. 39 mm. × 2.3. Animal shows no hint of any pigment or structural changes which characterize the incipient metamorphic period. Homogeneous yellow-brown larva.

From a study of forty Amblystoma punctatum it was evident that in a great majority of cases these pigment changes preceded the process of tail atrophy from one to eleven days. In seven individuals pigment changes and tail atrophy occurred simultaneously. Three animals not recorded in table 1 must be mentioned here. Two of these were preserved for histological study on the first and second days of tail atrophy, at which times no pigment changes could be determined. The third individual showed pigment changes one day after tail reduction.

These variations shown in the period of initial pigment changes were more marked in individuals taken from the field in the late larval stage and studied in the laboratory through the transformation period than in the cases of animals which had been raised in the laboratory from the blastula and fed the same rich diets and kept under identical temperature conditions. Moreover, animals from the same nests showed marked uniformity in pigment details. It is, of course, true that pigmentation in all animals shows more variation than any other structural factor. Its responses to light are well known and differences in the rate and intensity of its changes are to be expected. It is believed, however, that as the observer becomes more and more familiar with these variations and as more studies confined to animals raised in the



Figs. 4 and 5 Amblystoma punctatum. 39 mm. × 2.3. Ten days post initial skin shedding. Typical change which may occur about fifteen days after that of figure 1. Pattern of metamorphosed adult.

laboratory and kept under identical conditions (including studies of individuals originating from the same nests), are made, there will appear an increased uniformity in the sequence of events of this early pigment-migration period.

The fact which has thus far been established, however, is the existence of an incipient metamorphic stage which may be seen from a study of changes in pigment pattern. Furthermore, it has been shown that in a very large majority of cases (thirty out of forty) it precedes any other hint of approaching metamorphosis. These pigment changes which appeared before any tail or gill atrophy could be distinguished are interpreted as marking a true incipient metamorphic stage for the following reasons:

TABLE 1

Initial pigment changes and tail atrophy: Amblystoma punctatum

HISTORY OF ANIMAL	TOTAL	NUMBER OF ANIMALS SHOWING INITIAL TAIL ATROPHY ON SPECIFIED DAY OF PIGMENT CHANGES										
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th
Raised in		2	2		4	–		2				
laboratory	15		1[1]	2[1]			1[1]		1[1]	–	–	–
Obtained		5	2	1	1	3	–	1	2	1	1	2
from field	25		1[1]		3[1]				2[1]			
Totals	40	7	6	3	8	3	1	3	5	1	1	2

[1] Number of individuals preserved for cytological study on specified day of pigment changes before tail atrophy had appeared.

1. Histological studies of the integument of this period showed early formation of acinous glands as small groups of cells in the stratum germinativum. This condition was one used by Wilder to determine the 'incipient metamorphic' stage in Eurycea. The adult acinous gland was interpreted by her as a morphological constituent of the typical land integument.[2]

2. Histological studies of the thyroid gland of this period showed evidences of hyperactivity.

---

[2] It should be noted that 32-mm. Necturus embryos show anlagen of multicellular skin glands which sink into the dermis and form a lumen in the 37-mm. stage. (Eycleshymer and Wilson, '10; Dawson, '20.)

It might be questioned whether pigment pattern in the skin is not a self-differentiating system. Uhlenhuth showed, in 1917, however, by transplantation experiments with larval skin grafts of Amblystoma punctatum that the formation of the pigment pattern is dependent upon a metamorphic factor, since the graft metamorphosed only in case the host did. Therefore it seems that the initial pigment changes described in this report may be interpreted as part of the metamorphic process.

Tail atrophy is the next event in the series of changes. Observations on twenty-seven individuals, some of which were obtained from the field and others raised in the laboratory from the blastula stage, showed that tail atrophy occurred from one to three days prior to skin shedding, except in two cases in which the phenomena occurred simultaneously. Table 2 records the time relation between tail atrophy and initial skin shedding in eighteen animals all of which were obtained from the field in August and observed in the laboratory as they passed through the metamorphic process. In this group tail atrophy always preceded skin sloughing and in the majority of cases the initial molt appeared on the second and third days of tail reduction.

TABLE 2

Tail atrophy and skin shedding

ANIMAL	TOTAL	NUMBER OF ANIMALS SHOWING FIRST SKIN SHEDDING ON SPECIFIED DAY OF TAIL ATROPHY							
		1st	2nd	3rd	4th	5th	6th	7th	8th
A. punctatum	18	–	8	7	3	–	–	–	–
T. viridescens	17	–	–	5	5	4	2	1	–

Gill atrophy in its earliest phase is the most difficult to determine of all the metamorphic phenomena, and for this reason its use as a reliable criterion was omitted. The first suggestion of gill reduction was sometimes seen by an increased pigmentation and a curling of the filaments. The difficulty of determining this with certainty became evident when it frequently happened that a positive report for this phenomenon on one day was followed by a negative one on the next. Histological verification of this point did not especially aid in the determination of the first hint of gill atrophy. The degenerating gill showed numerous dark chromatophores in the connective-tissue spaces of the filament, and the disintegrating epithelial cells which had lost their nuclei contained deep-staining acid and basic products. In a series of studies of Amblystoma opacum, no case with this histological picture was found before indications of it could be determined by microscopic examination of the living animal.

The analysis of the metamorphic process which has been described in Amblystoma punctatum may be summarized as follows:

Incipient metamorphic stage. (Generally preceded the metamorphic stage from four to eight days.) First appearance of dark pigment areas. Anlagen of acinous glands. Beginning of hyperactivity of thyroid.

Metamorphic stage. Tail atrophy. (Preceded skin molting one to three days.) Mature acinous glands. Disappearance of Leydig cell. Skin shedding. Adult pigment pattern complete.

PLATE 1

EXPLANATION OF FIGURES

Amblystoma punctatum, no. 332. 41 mm. × 3. Early pigment changes of incipient metamorphic stage.

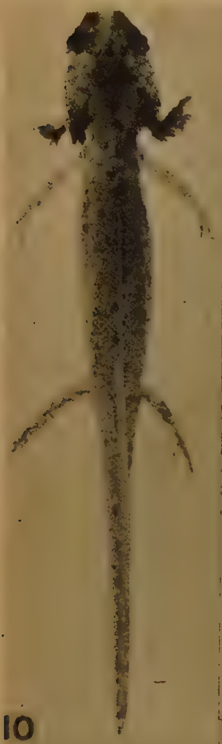
10, 14 Photographs taken 3/7/29. One day post pigment changes. No atrophy of tail. No skin shedding.

11 Photograph taken 7/7/29. Five days post pigment changes. Note dark areas are similar, though larger. No tail atrophy. No skin shedding. No gill atrophy.

15 Photograph taken 8/7/29. Six days post pigment changes. First hint of tail atrophy. No skin shedding.

12 Photograph taken 9/7/29. Seven days post pigment changes. First day of skin shedding. Tail shows increased atrophy. Right gill shows early atrophy.

13, 16 Photographs taken 10/7/29. Eight days post pigment changes. Increased tail atrophy. Metamorphic process well advanced. Right gill shows increased reduction over that of left.



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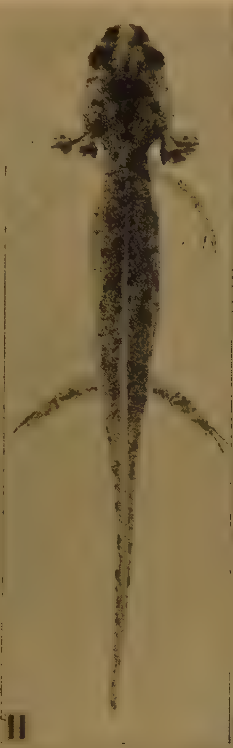

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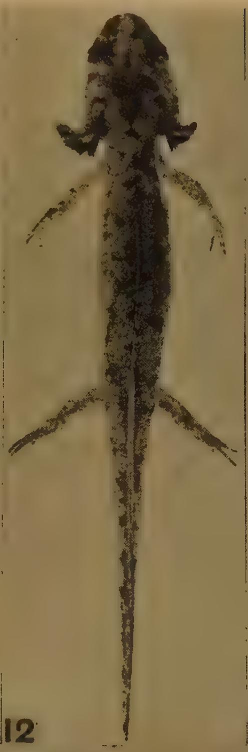

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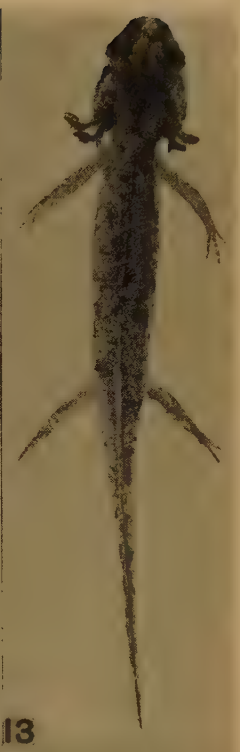

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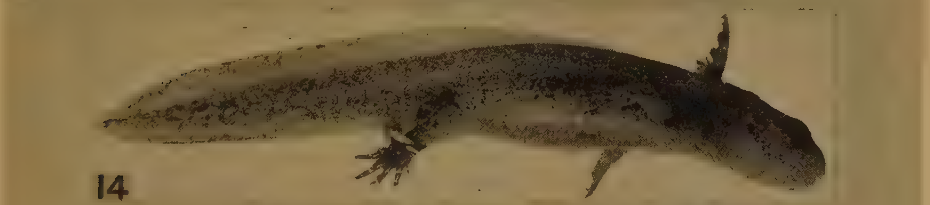

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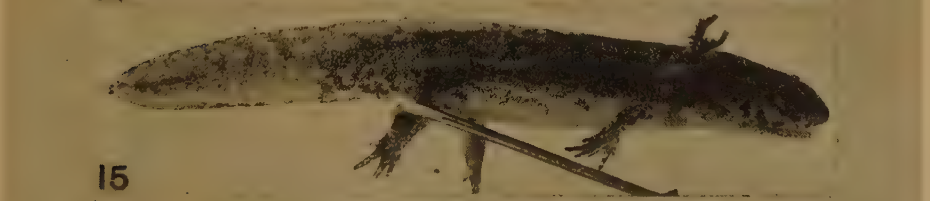

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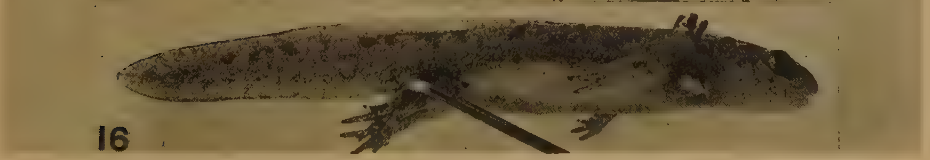

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PLATE 2

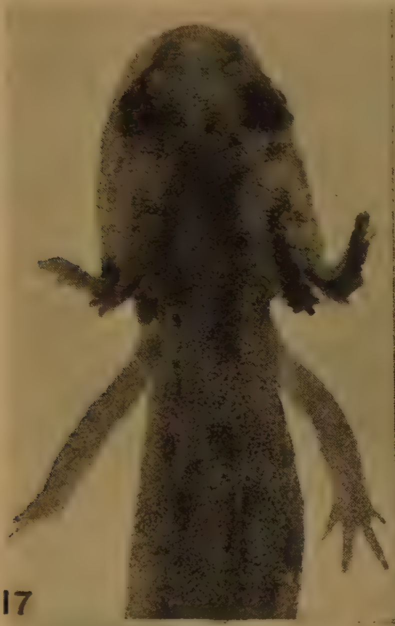
EXPLANATION OF FIGURES

Amblystoma punctatum. Illustrations show how the early pigment pattern becomes extended and more definite until the final adult picture is suggested.

17 Early pigment changes and beginning tail atrophy. No tail atrophy. No skin sloughing.

18 Same animal as figure 17. Photograph taken four days later. Initial pigment changes extended and clearer. Four days post skin shedding.

19 Another animal ten days post initial skin shedding. Pigment pattern of adult suggested.



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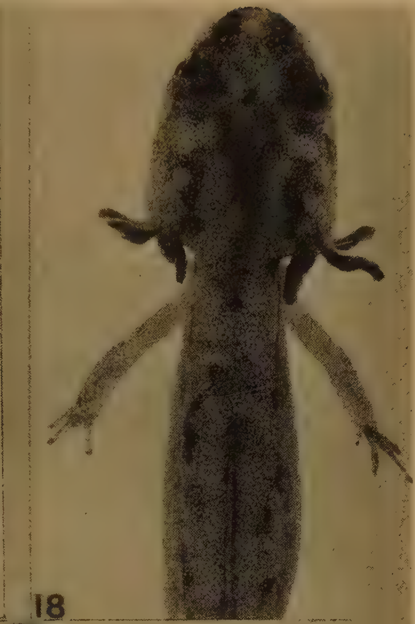

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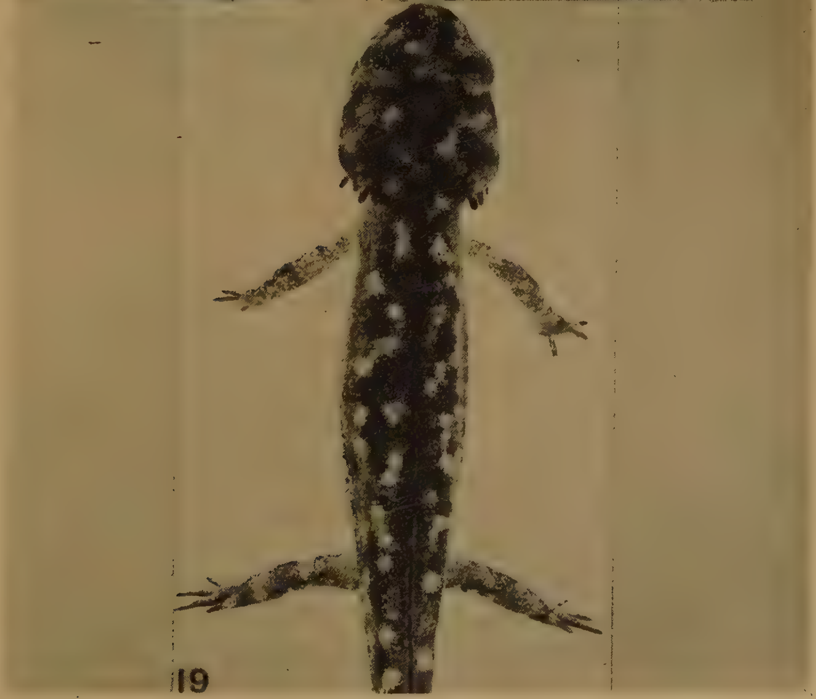

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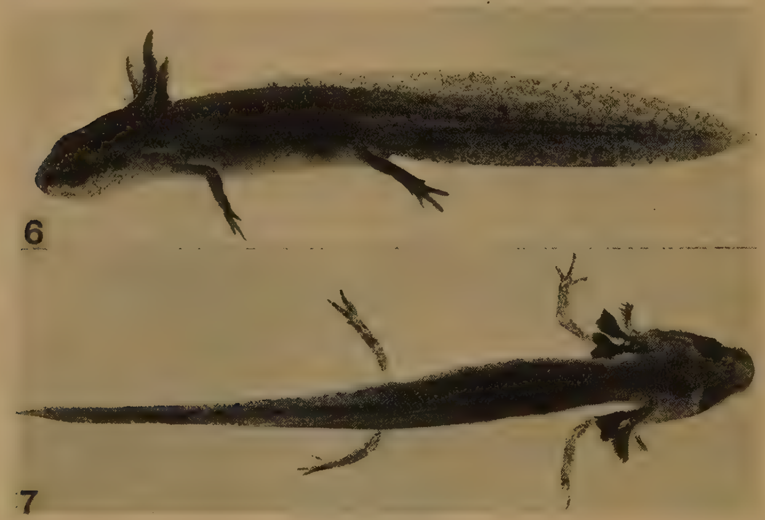
TRITURUS VIRIDESCENS

The sequence of events in the metamorphic process of Triturus viridescens is identical with that described for Amblystoma punctatum. The entire process occurs more slowly, however, and as a result each event is more easily distinguished than is possible in the case of Amblystoma punctatum.



Figs. 6 and 7 Triturus viridescens. About × 2.4. Homogeneous light yellow larva.

Pigment changes which first indicate approaching metamorphosis occurred in one of two ways: 1) the homogeneous light yellowish larva (figs. 6 and 7) became uniformly dark brown in color and the discrete black spots which appeared a few days later were often difficult to see. 2) Discrete black spots appeared upon a light-colored larval animal and stood out with great clearness as soon as they were first formed (pl. 3).

Observations upon pigment changes have been made on twenty-three Triturus viridescens. The time relation of this initial metamorphic phase and tail atrophy is recorded in table 3. In this group tail reduction never occurred prior to a change in the animal's pigment pattern and in fifteen cases changes in coloration preceded changes in the tail from at least one to eight days. In five of the seven cases in which the two phenomena occurred simultaneously it was recorded that the type of change was that which has been described as a general darkening of the animal. It is obvious that the beginning of a change of this type is much more difficult to determine than is the appearance of discrete black spots. The actual time, therefore, when this type of pigment change appeared may have been earlier than that recorded.

TABLE 3

Pigment changes and tail atrophy: Triturus viridescens

SOURCE OF ANIMAL	TOTAL	NUMBER OF ANIMALS SHOWING INITIAL TAIL ATROPHY ON SPECIFIED DAY OF PIGMENT CHANGES								
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th
Taken from field in August –	23	7	1	2	3	1	1	–	1	1[1]
		1[1]	3[2]	1[1]					1[1]	

[1] Indicates number of animals preserved for cytological study on specified day of pigment change at which time no tail atrophy had begun.

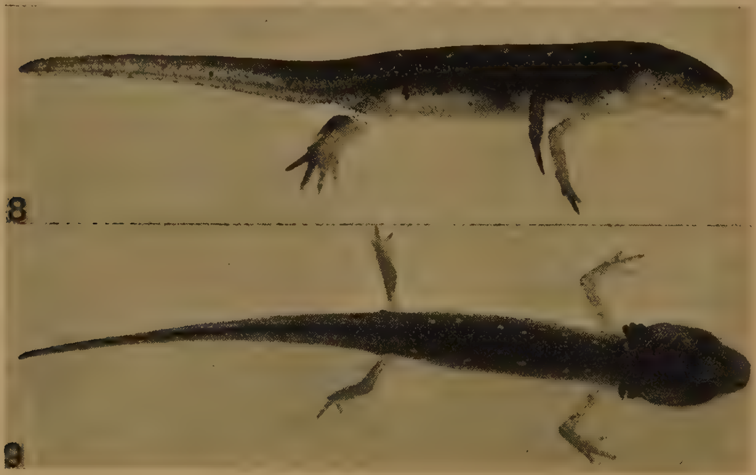
[2] Indicates number of animals in which tail atrophy occurred on day specified or one or two days later.

From a study of the above group of animals it is evident that an initial metamorphic stage can be determined in the nature of pigment changes and that these appear before any tail or gill atrophy can be seen. As in the case of Amblystoma punctatum, acinous glands are forming in the integument during this incipient stage and the thyroid gland shows hyperactivity.

An individual in this early pigment stage, illustrated by figures 20 and 23, was photographed by Pope ('24, pl. 44, fig. 5) and cited by Wilder ('25). Both these authors interpreted the animal of this photograph as a "young larva showing full development of gills and tail." The same animal was photographed by Pope six days later (pl. 44, fig. 7) and printed with the caption, 'metamorphosis already begun.'

These earliest pigment changes are those from which the final adult pattern is derived (figs. 8 and 9). Plate 3 shows an animal (figs. 20, 23 and 21, 24) in which the black spots of the adult are becoming increasingly clearer. Figures 22, 25 on the same plate show another individual in which the discrete, light yellow spots represent the future red spots of the adult.

Tail atrophy appeared as the next event in the metamorphic process. In the cases of seventeen Triturus obtained from the field and studied in the laboratory as they passed through the metamorphic process, tail atrophy preceded skin shedding from two to six days. The majority of the animals shed initial skin on the third and fourth days of tail reduction. Table 2 pictures the time relationship of these two phenomena as they occurred in Triturus and Amblystoma and indicates that initial skin shedding occurred later in relation to tail atrophy in Triturus than it did in Amblystoma and that the two phenomena were associated over a longer period of time than was the case with Amblystoma.



Figs. 8 and 9 Triturus viridescens. About × 2.4. Adult pigment pattern becoming established, a change which may take place within fourteen days after stages shown in figures 6 and 7.

It is clear that a pigment change in a homogeneously colored larval animal marks an incipient metamorphic stage in Triturus and also in Amblystoma. The sequence of events established for the true metamorphic period in Amblystoma holds for the same stage in Triturus. The rate of changes in the latter species is slower than in Amblystoma, and for this reason Triturus is recommended as valuable material for study of the metamorphic process.

DISCUSSION

The initial pigment changes described in this paper are interpreted as marking incipient metamorphic periods—a position contrary to the opinion held by Wilder ('25):

> It is mainly during the latter part of the metamorphic process that those changes in coloration occur which result in the characteristic pattern of the adult stage, so different from that of the larval period, although derived directly from it, as Banta and McAtee ('06) have shown to be the case with the adult coloration in general in the urodeles. Development of adult coloration, however, does not hold so close a correlation with other metamorphic processes as was formerly supposed, and it has been recently shown by Gutman ('23) that in the case of experimentally induced transformation in Eurycea larvae no change in color from that of the controls occurs.

On the other hand, Ingram ('29) reported from his experimental work on anuran larvae, which he caused to metamorphose by repeated transplantation of larval pituitaries, that:

> The first evidence of the effective action of the grafts was in the extreme darkening of the skin soon after operation, due to the presence of pars-intermedia tissue in the transplanted material. . . . Increased growth of the limbs (index of metamorphosis in anurans) was noticeable within six days after the first graft.

Pigment changes and initial tail atrophy are recognized as better criteria than skin sloughing when the object is to obtain material for cytological study during the earliest recognizable metamorphic phase.

PLATE 3

EXPLANATION OF FIGURES

Triturus viridescens. 29 mm. × 3. Early pigment changes of incipient metamorphic period. Discrete black spots clearly discernible.

Figures 20, 21, 23, and 24 are serial pictures of same animal.

Figures 22 and 25 are serial pictures of another animal.

20, 23 Photographs taken 20/7/29. Discrete black spots beginning to appear. No tail or gill atrophy. No skin shedding.

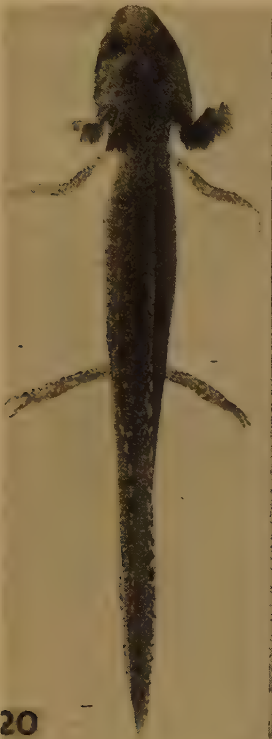
21 Photograph taken 26/7/29. Discrete black spots more evident. First day of tail atrophy. No skin shedding.

24 Photograph taken 29/7/29. Three days post tail atrophy. Gill reduction evident. Skin has been shed.

25 Photograph taken 25/8/29. Eight days post pigment changes. Five days post tail atrophy. One day post skin shedding.

22 Photograph taken 30/8/29. Thirteen days post pigment changes. Ten days post tail atrophy. Six days post skin shedding.

The discrete light spots represent the future red spots of the adult.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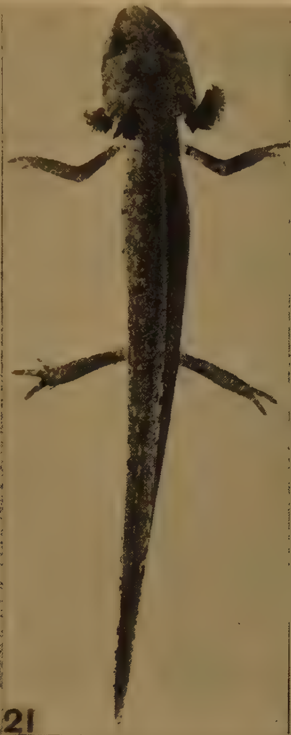
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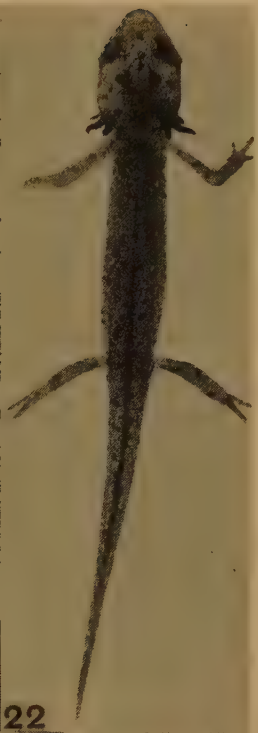
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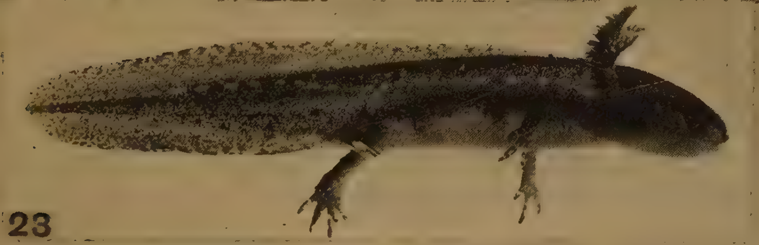
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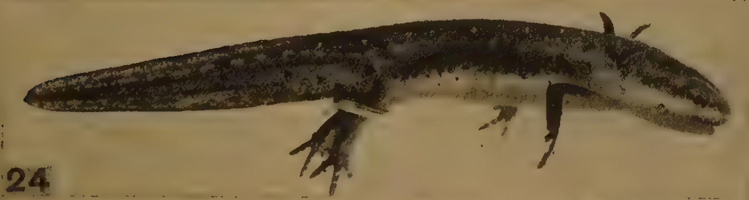
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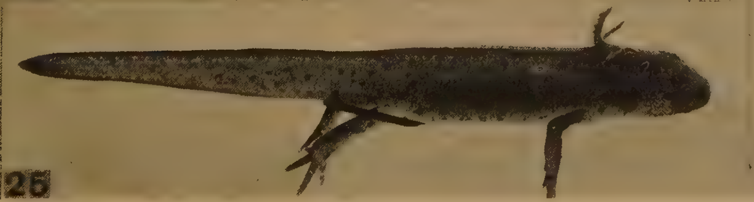
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Data reported in this paper establish the fact that within the same species individual variations in the rate of the metamorphic process may exist. These variations may be due to: 1) individual differences in the rate of thyroid activity or, 2) individual differences in the susceptibility of tissues undergoing change, or to a combination of these factors. DeBeer ('26) offered these hypotheses to explain differences in the metamorphic rate of different anuran species. Material for cytological study dated according to methods suggested in this paper furnishes detailed information concerning the condition of the animal in relation to the metamorphic process. It also permits of detailed comparison of individual cases which may aid in the interpretation of individual differences. For instance, the cytological examinations made on material from cases no. 357 and no. 356 give opportunity for the study of two individuals in which the metamorphic rate has been approximately the same up to initial skin shedding.

Case no.	*Pond*	*Fixed*	*Changes recorded*
356	3/8/29	13/8/29	Four days post pigment changes One day post tail atrophy No skin shedding
357	3/8/29	13/8/29	Four days post pigment changes One day post tail atrophy First day of skin shedding

Two other cases illustrate material in which the rate of change has been different throughout.

Case no.	*Pond*	*Fixed*	*Changes recorded*
388	10/8/29	7/9/29	Eight days post pigment changes Four days post tail atrophy Two days post skin shedding
384	10/8/29	3/9/29	Five days post pigment changes Five days post tail atrophy Two days post skin shedding

CONCLUSION

1. Amblystoma punctatum and Triturus viridescens differ in the metamorphic process, *a*) in the picture of the initial pigment change; *b*) in the rate at which the process as a whole takes place.

2. Amblystoma punctatum and Triturus viridescens exhibit the same sequence of events: 1) initial pigment change; 2) tail atrophy; 3) skin shedding.

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AN EXPERIMENTAL STUDY OF THE ISLET CELLS OF THE PANCREAS IN VIVO

JAMES L. O'LEARY
Anatomical Laboratory, Washington University School of Medicine, St. Louis

FIVE FIGURES

The recent renewal of attempts to examine directly the tissues of the mammalian body while they are actually functioning has been made with different methods by Sandison ('28), Covell ('28), and Markee ('28), studying vascular phenomena, glandular activity, and the female sex cycle, respectively. Their success has emphasized the importance of studies of this kind in the solution of problems which remain unsolved after the diligent study of fixed preparations by many investigators. To look directly into the body and to ascertain just what is happening is certainly a long step in advance.

The endocrine glands offer a particularly fruitful field for such investigation, because our knowledge of cellular activity is confined to the results of reduction in amount of tissue by surgical means, diet changes, and the introduction of drugs or hormones. In all of these glands the probable existence of intracellular secretory antecedents has been postulated, but their sure identification as well as the mechanism of their removal from the cells remains to be determined. A good point of departure is afforded by the islet cells of the pancreas, because they can be examined in the living state by Covell's ('28) method and in view of the fact that the action of the islet hormone in vivo is better understood than that of any other endocrine gland, though with all of them we could wish for more detailed information.

A study designed to determine the source of insulin and its mode of elimination is fraught with obvious difficulties: 1) The specific granules of the islet cells, which, by analogy to the zymogen granules of the acinar cells, have been considered the probable intracellular antecedents of insulin, have roughly a mass of but one-thousandth that of mature zymogen granules. 2) It would be necessary to detect their movement not into empty duct lumina, but into capillaries through which blood seems to flow very rapidly in view of the high magnification used, though in reality it passes by the cells comparatively slowly. 3) The brilliant light necessary for a detailed study of the cells, the anesthesia used, and unavoidable operative trauma may inactivate these highly specialized endocrine cells. 4) Attempts to alter the appearance of islet cells in fixed preparations by various experimental procedures have failed with but few exceptions.

Allen ('14 and '21) and Homans ('14 and '15) have reported hydropic degeneration in islet cells resulting from functional overstrain produced by the removal of from five-sixths to nine-tenths of the pancreas in cats and dogs. Homans, by the application of the cytological techniques devised by Lane and Bensley, was further able to demonstrate a series of changes beginning with a reduction of specific granules and an increase in the mitochondrial content of the B cells in cats exhibiting intermittent glycosuria and terminating in hydropic degeneration of B cells in those animals which showed severe and permanent diabetes. Intermediate stages were characterized by the presence of isolated vacuoles embedded in the cytoplasm of the B cells and by vacuolar degeneration of the B cells of scattered islets. Both Allen and Homans were particularly successful in demonstrating that in animals in which the amount of pancreatic substance had been reduced in this way a high carbohydrate diet resulted in severe and lasting diabetes with hydropic degeneration of the B cells of the islet remnants.

In view of the lack of success experienced by other investigators, the problem of insulin secretion presents two alternatives: first, that the islet cells respond to the demands of carbohydrate metabolism by the liberation into the blood vessels of their specific granules individually or en masse; and, secondly, that their specific granules are converted into an intermediate, labile, and transient product before elimination.

The application of Covell's method to an experimental analysis of the nature of the secretory process in islet cells brings new information regarding the appearance and distribution of the specific granules and other microscopically demonstrable materials of normal living cells, their reaction to vital dyes, and, finally, data concerning the behavior of these cells in the living animal during the compensation necessary in altered carbohydrate metabolism in response to agents calculated to accelerate secretion. The purpose of this paper is to report briefly the results of such an experimental investigation.

MATERIAL AND METHODS

The ease of adapting Covell's ('28) method of preparing the living pancreas of the white mouse for observation prompted the use of this animal for all the experiments. Numerous strains of white mice were examined before two strains were found in which the pancreas was so constituted that it was easily spread in a very thin layer between the slide and cover-slip for examination, and prominent islets of the interstitial category of Bensley ('11) were commonly observed in close association with the larger vascular channels. All the precautions indicated by Covell were carefully followed, and the reader is referred to his excellent description of the method used.

Mice of the selected strains weighing between 13 and 20 grams gave the best results, and, in most of the experiments, preparations were made without noticeable loss of blood and with very little trauma. Ringer's solution warmed to 37°C. was used as a mounting medium for all preparations, except three controls which were mounted in homologous serum. The lack of obvious differences between islet cells of pancreases mounted in homologous serum and in Ringer's solution and the time required for the collection of blood rendered the routine use of serum impractical.

A Spencer Mazda microscope lamp gave sufficient illumination, provided that the preparation was not too thick. Intense illumination does not compensate for thick mounts and may be harmful to the cells. To avoid unnecessary injury from this source, the iris diaphragm was closed down until the light just sufficed to permit the identification of the specific granules as entities and to render the capillary margin of the cells distinct.

The possibility had to be tested that the anesthetic used might lessen the activity of the islet cells. To reduce any errors which the action of the anesthetic might introduce to a minimum, parallel experiments were performed in which amytal (sodium salt of isoamyl-ethyl-barbituric acid), urethane, and light ether anesthesia were employed. The difficulty of maintaining even anesthesia with ether during observation and the low blood pressure which is apt to occur under urethane have made amytal the most dependable anesthetic. It is of added advantage that amytal but slightly affects the blood sugar level (Page, '23, and Britton, '25), whereas both urethane and ether induce significant hyperglycemias.

The question arises, despite the precautions taken to insure normal functioning of the islet cells under observation, whether the long periods of removal from the body may not induce pathological change. A rapid circulation of blood has been maintained in the islets of six control mice kept under urethane anesthesia for a period of six hours with intermittent observation. Figure 1 illustrates the appearance of one of these islets after fixation in aqueous chrome sublimate and staining with hematoxylin and eosin. A comparison with figures 1 and 2 of Lane ('07) shows no deviation from the normal.

STRUCTURE OF THE LIVING ISLET

1. Arrangement of the cells

The islets in mice are composed of spherical or ovoid masses of closely packed cells. In size they vary from very small size to 1 or 2 mm. in diameter. Their relation to the ducts and blood vessels in the pancreas of the guinea-pig has been so well discussed by Bensley ('11) that further comments upon their position seem unnecessary, since substantially the same relations obtain in mice.

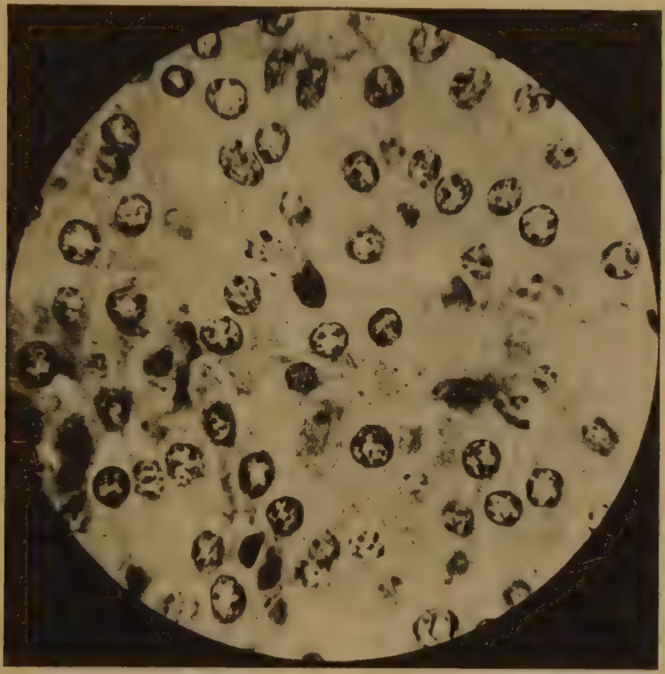


Fig. 1 Mouse 17. Photomicrograph of a 4 μ section of mouse pancreas (hematoxylin and eosin stain). The islet shown had been under continuous observation for six hours. Note the normal appearance of its cells. Zeiss apo. 1.3 n. ap. × 850.

A profusion of sinuous capillaries penetrates each of the macroscopic islets. Their walls are thin and the continuity of endothelium throughout is difficult to demonstrate. Numerous endothelial cells, their processes drawn out to exaggerated length, intervene between the free borders of the islet cells and the capillary channels; but it also appears that islet cells may project directly upon the capillaries without the intervention of an endothelial membrane. In fact, narrow intercellular canaliculi sometimes communicate with the capillaries.

Kühne and Lea ('82) first noted that the capillaries of islets appeared to be wider than those of acini, and their impressions were verified from time to time by other investigators. Because no significant differences in width were apparent in living preparations, measurements were made with a filar wheel micrometer of sixty-seven islet and fifty-two acinar capillaries. The mean of the former was 5.5 μ, with a standard deviation of 1.26; the mean of the latter, 5.81; standard deviation, 1.29. Seventy-five per cent of the islet capillaries fell within the range of 5.0 to 7.5 μ, 81 per cent of the acinar capillaries between 4.5 and 7.5 μ. Therefore, no significant difference in width between the capillaries supplying the exocrine and endocrine tissues is indicated.

Through the application of Janus green B intravitam it is possible to demonstrate the rich nerve supply of the islets electively colored. It is made up, as described by Gentes ('02) and deCastro ('23), of a peri-insular network with ramifying branches which penetrate the interior of the islets.

Patent lymphatic channels have never been observed in the interior of islets, despite Biedl's ('07) theory that insulin is removed by lymphatics. However, vessels which in their appearance suggest lymphatics do occasionally course across the surface of an islet.

2. *Structure of individual cells*

The polymorphism of the islet cell has been a subject commented upon by numerous investigators (Cowdry, '23). In living preparations, columnar cells abutting at one or both poles upon a capillary predominate; polyhedral cells, with two or more surfaces bathed by blood, are frequently situated at a confluence of capillaries; and by no means unusual are roughly polyhedral or columnar cells differing from the first-mentioned variety in that their longest axis lies parallel to the adjoining capillary. The actual surface area of a cell bathed directly by blood is, therefore, not easily decided by appearances, for while observations in a single plane indicate that two or even three surfaces may be in contact with capillaries, the relation of cells to capillaries coursing above and below them is not easily made out.

Each cell is bounded on all sides, including those next the capillary and others separating it from adjacent cells, by a clear homogeneous membrane. The cell membranes appear as granule-free slits between the cells, while upon the capillary surfaces they vary considerably in thickness from cell to cell. These may either be as thin as the intercellular membranes and scarcely recognizable, or appear as a broad corrugated flange outlining the cell. The latter condition is most often observed at the bends of capillaries. At times it is so pronounced that it lends a pathological appearance to the cell.

The round or slightly oval nucleus is excentrically placed in the cytoplasm, sometimes in contact with the capillary surface of a cell; but in polyhedral cells which are situated at the confluence of capillaries, the nucleus is usually located at the point farthest removed from the capillaries.

Under the ordinary illumination which we have used, the nucleus appears to be optically homogeneous and differs from the cytoplasm only in the absence of specific granules and in refractive index. With more intense illumination the characteristic lumps of chromatin seen in fixed preparations can be readily outlined.

3. *Cytoplasmic materials*

a. Specific granules. The greater number of living islet cells exhibit the dense packing of specific granules described by Bensley ('11). In others, however, the granules appear to be less closely aggregated and of a lower refractive index, scarcely distinguishable from the matrix of cytoplasm. It is probable that Laguesse ('10) had in mind these differences when he attempted to distinguish two types of isolated fresh cells. But it must be remembered that the only way that they may be judged by the criteria which Lane ('07) and Bensley have set forth for the differentiation of alpha and beta cells is by accurately localizing the cells of either of the two granular types in an islet; then, after fixation and staining by the techniques prescribed by the latter authors, it would be necessary to ascertain in fixed preparations whether all cells of one or the other granular types belong to the category of alpha or of beta cells. The difficulty of such a determination in a closely packed group of cells is evident. The alternative, therefore, remains that the cells which are less granular belong to the type first described by Bensley as 'indifferent cells.' An indifferent cell, in the strict sense of the term, usually polyhedral in shape, is but rarely observed in the islets of mice. The cytoplasm is utterly devoid of specific granules, even though its neighboring cells show the characteristic granule pattern. The other inclusions, to be described, are not lacking, and their disposition appears identical with that observed in the typical granular cells.

b. The canals of Holmgren. Bensley ('11) has reported a peculiar arrangement of clear-looking spaces in isolated but still living islet cells following the aortic injection of neutral red (1:10,000 in normal saline). He says, "Owing to the small size of the granules it is possible to observe in the still living cell the outline of the canals of Holmgren, which appear as colorless spaces among the deeply stained granules." Cowdry ('24) has justly called attention to the necessity of confirming such observations in islets with the circulation intact, for "the mere act of taking living cells from the body and bringing them under the microscope may . . . . initiate changes in the cytoplasm . . . . responsible for their appearance."

Bensley's designation of the non-granular spaces in the living islet cell as 'canals of Holmgren' was occasioned by their correspondence to similar clear canals which he was able to demonstrate in islet cells of the guinea-pig after fixation in a mixture of formalin, potassium bichromate, and corrosive sublimate and staining with basic dyes, thus confirming the observations of Holmgren ('04) and Tschassownikow ('06). In a recent contribution (Owens and Bensley, '29), he adheres to his earlier observations ('10) supporting the theory that this canalicular apparatus of the animal cell is the homologue of the vacuolar system, or, in a later terminology, the vacuome (P. A. Dangeard, '19) of the plant cell. His observations upon the dermatogen and underlying cells of the growing onion root tip show transitions from a typical canalicular apparatus in the former to a system of vacuoles in the latter; and he has further indicated that he accepts the canalicular form as the prototype in the living plant cell as studied in freshly expressed homologous juice.

A system of spaces approximately identical with those described by Bensley is a constant constituent of the cytoplasm of islet cells of the mouse as seen in the living state with circulation intact. In general, the canals are situated in the greatest expanse of cytoplasm. They may appear at either the capillary or the extracapillary pole of those columnar cells which apparently abut at but one pole upon a capillary. In roughly columnar cells, one of whose long surfaces rests upon the margin of a capillary, they are similarly situated relative to the cell as a whole, but, of course, their relation to the feeding and eliminating capillary is changed. They are equidistant from the converging streams of blood in those polyhedral cells whose several surfaces are in contact with capillaries.

Bensley, studying fixed tissue, admitted the confusing relation of the canalicular apparatus to vascular and non-vascular surfaces of the cell, but discredited the idea of the existence of indifferent polarity in favor of one of strict extracapillary polarity. His statement, "while at first sight it seems as if the networks were located indifferently on the capillary or the other side of the nucleus, when a column of cells composed of two layers in thickness is cut through the center, the canals are invariably found on the side of the nucleus furthest from the capillary,'' may be quite commonly verified, but as has been emphasized, this limited state of affairs by no means disposes of the cell-vascular relationships which may be encountered in living islets.

The closed systems of canals which I have studied are composed of optically homogeneous material distinguishable with favorable illumination by their refractive index and the absence of specific granules or other cell inclusions. No interphase membrane separating the canals from the surrounding cytoplasm can be distinguished with even the 2.0-mm. 1.4-n.a. lens. Occasional exceptionally favorable preparations have made possible the comparison of unstained mitochondria with the canalicular system, side by side in the same cell. Under these conditions, which render differences in illumination negligible, the mitochondria are optically denser than the material composing the canals.

In islet cells having abundant cytoplasm, which are in the majority, the system of canals is usually arranged as a diffuse network, of which only very elongated slender spaces may be distinguished at a single level of focus. However, careful focusing leaves no doubt of the continuity of the parts. The segments near the nucleus are often somewhat wider than the remainder of the apparatus and might with justice be interpreted as similar to the spaces which Tschassownikow ('06) has called 'nuclear caps' in the islet cells of the rabbit and which he referred to the category of 'trophospongia.' Extensions from this portion of the canal system around the nucleus, which Bensley has noted in fixed preparations, are of frequent but not constant occurrence.

Occasionally in cells with abundant cytoplasm, and so frequently in smaller cells that it may be regarded as the rule in their case, the larger part of this canalicular apparatus forms a dense net in close proximity to the nucleus. At the proper level of focus the individual canals of this dense network are distinguishable, the interstices occupied, as in the more diffuse form, by the cytoplasmic matrix containing the embedded specific granules. When the cell under observation is slightly out of focus, however, the region occupied by the canals may appear as a single large vacuole.

Characteristic of the appearance of the canals as heretofore described are: 1) The continuity of parts. 2) The rounded contours which Cowdry ('24) has recognized in fixed tissue as an important criterion for distinguishing the true canals of Holmgren from clefts in the tissue produced through faulty technique. 3) The fact that the changes in diameter from part to part are usually gradual, rarely abrupt. 4) The component material is optically homogeneous. But some cells are to be found in every islet in which material optically identical with that composing the canals appears to be distributed not in canals, but rather in discrete oval or elongated spaces. The most careful focusing fails in such cases to reveal continuity of parts, and while there may be a tendency toward alignment of these unstained masses, this question is so obviously one of conjecture that it is purposely eliminated from consideration.

c. Lipoid droplets. Small droplets of high refractive index and comparable in size to the smallest zymogen granules of the acinar cells are encountered in every living islet cell. They most frequently impinge upon the capillary border, but are occasionally found well in the interior. Five is the largest number that I have counted in a single cell. These droplets are probably identical with the 'lipoid corpuscles' which Saguchi ('24) identified in the islet cells of the frog after Ciaccio's ('11) method and likewise after fixation in osmic acid and the subsequent application of the Fauré-Fremiet technique.

d. Watery vacuoles. These are also to be observed within the living islet cells in proximity to the capillary margins. They are so tiny as to be upon the border line of visibility, and the difficulty of certainly identifying them renders futile any appraisal of their distribution or constancy.

THE REACTIONS OF LIVING ISLET CELLS TO VITAL DYES

1. Neutral red (Grübler, lot no. 0526)

Following the technique recommended by Covell ('28) and Covell and Scott ('28) for the study of neutral-red-stainable vacuoles in gland and nerve cells, amounts varying from 0.25 to 1.0 cc. of 1:200 and 1:5000 solutions of the dye in distilled water and in normal saline were injected intraperitoneally at periods from one-half to eight hours preceding the examination of the pancreas. The criterion accepted for optimum staining with neutral red was the appearance of close clusters of colored vacuoles occupying the zone of young zymogen granules in the acinar cells (Covell).

In the islet cells the specific granules, which are stained by Bensley's supravital method, rarely color with neutral red; the dye is confined to several vacuoles comparable in size and disposition to Saguchi's 'lipoid corpuscles.' But occasionally in young mice in which the pancreas is quite thin and the islets advantageously situated for direct contact with the dye, the specific granules can be colored by neutral red. The appearance of the cells in which the granules are thus stained deviates in no particulars from Bensley's previous description.

The canalicular apparatus, which, as has been mentioned, is demonstrable in the living unstained cell, is not specifically colored by neutral red. Often, in those experiments in which only the vacuoles have been colored by the dye, the canals and the colored vacuoles may be defined in the same cell (fig. 3, *1* and *2*). When the specific cell granules are stained, the canals assume a faint tinge of red, but their staining is no more pronounced than that of the matrix of cytoplasm.

2. Janus green B (H. A. Metz & Co. and National Aniline and Chemical Co.)

Janus green B was found to be so toxic when introduced intravitam by the method employed for staining with neutral red that resort was made to another procedure. The dye, in concentrations of 1:2000 to 1:100,000 in normal Ringer's solution (37°C.), was allowed to drip upon the exposed living pancreas. When the desired stage of staining was reached, the dye was washed away with Ringer's solution and the pancreas mounted for observation.

In a successful preparation the mitochondria of both islet and acinar cells, the nerve fibers, and certain cells of the small arterioles and capillaries are specifically colored. Overstaining is often difficult to avoid. The best results are obtained before the zymogen granules, prozymogen, and the muscle fibrils of arterioles become also colored. It is important that the Janus-green solution come in contact with the islets in fair quantities. In the early stage of absorption, the mitochondria are selectively stained and appear as slender filaments, sometimes as granules scattered through the cytoplasm. In columnar cells the filaments are usually oriented parallel to the long axis of the cell and are more abundant in the circumnuclear region. In the next stage, which seems to be a kind of saturation of the mitochondria with the dye, the specific granules are deeply colored and mitochondria are not easily discerned. Sometimes the two degrees of staining may be observed in the same cell, one pole of which is favorably located for direct access to the dye, the other not. Figure 3, *3*, shows a columnar cell whose capillary pole is crowded by the intensely stained specific granules, whereas at the extracapillary pole only mitochondria and scattered specific granules are stained. When the specific granules have adsorbed the dye, an uncolored system of spaces always remains in the region of the cell occupied by the canals of Holmgren.

The staining of living tissue by Janus green B is not a transient and fluctuating phenomenon, as Bensley ('28) and others have observed to be constantly characteristic of the same dye when applied supravitally. Preparations stained judiciously, in which all the mitochondria were brilliantly colored, have shown no noticeable reduction of the dye after several hours of observation. Twenty-four hours following the staining of a pancreas, provided that the circulation of blood has not diminished and the light has been just sufficient to render observation clear, considerable fragmentation of numerous mitochondria has occurred and green-stained globules, indicating the formation of lipoid droplets (Scott, '16), have appeared. However, other mitochondria retain their filamentous shape and are no different than they appeared at the beginning of observation.

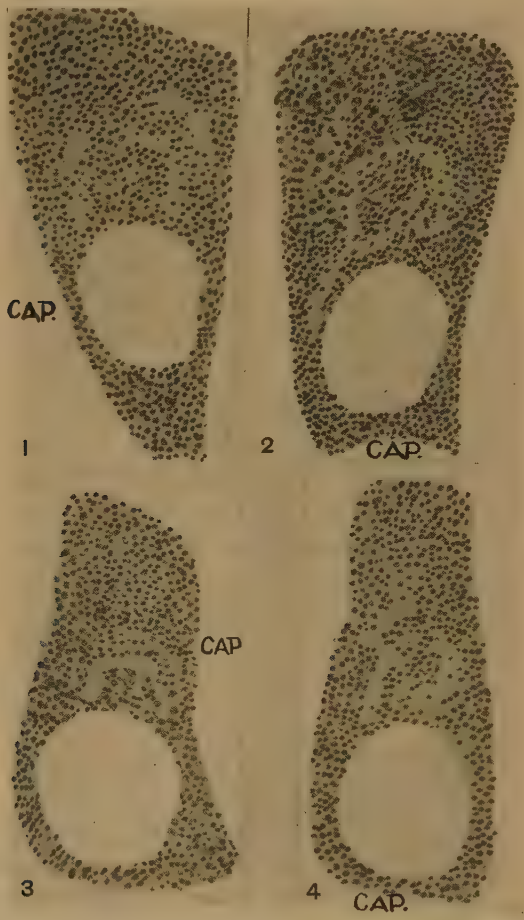


Fig. 2 Four islet cells drawn to illustrate the positions which the canals of Holmgren may occupy with reference to the capillary surface. *CAP*, capillary. Zeiss apo. 2 mm., 1.3 n.a. × 3000.

Both in acinar and islet cells the ability of mitochondria to retain Janus green is dependent, other factors remaining constant, upon the maintenance of the proper oxygen tension by the circulating blood. If the circulation be stopped in a localized area of the pancreas by pressing down upon the

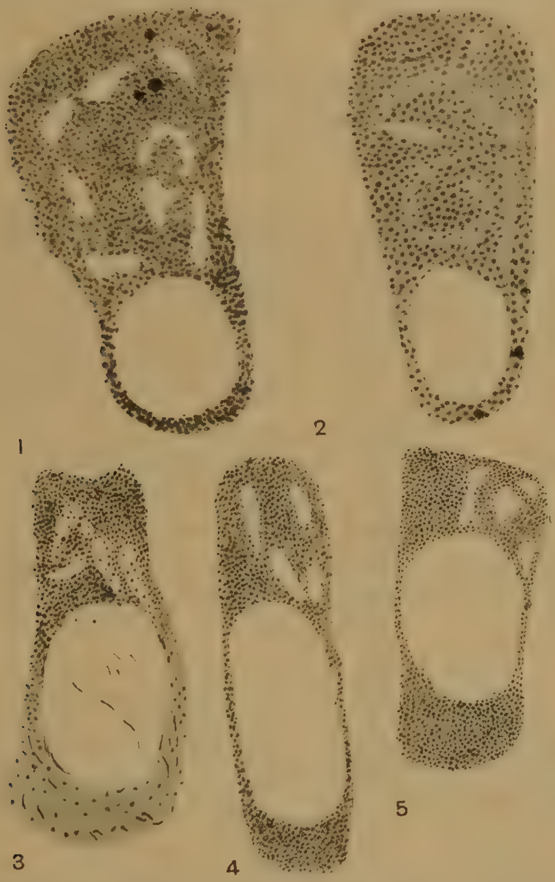


Fig. 3 Mouse 174. Reaction of living islet cells to vital dyes. *1* and *2* illustrate the position of the canals of Holmgren and neutral-red-stained vacuoles one and one-half hours following the injection of 1 cc. of 0.5 per cent neutral red intraperitoneally. *3*, *4*, and *5* show stages in vital staining by Janus green B. In *3* the capillary pole is crowded with intensely stained specific granules; at the extracapillary pole only scattered specific granules and mitochondria are stained. Neither neutral red nor Janus green B is segregated by this canalicular apparatus. Zeiss apo. 2 mm., 1.3 n.a. × 3000.

cover-glass, the dye immediately diffuses from the mitochondria into the surrounding cytoplasm, which then becomes diffusely stained. With renewal of the circulation, the dye is again concentrated by the mitochondria and the cytoplasm again appears unstained. The repetition of this experiment a dozen times within the space of as many minutes proves the accuracy of the observation.

Excessive light is to be avoided. Under its influence the circulation of blood rapidly diminishes, the mitochondria often permanently lose their affinity for the dye, and reduction proceeds.

The following other Janus dyes have been applied vitally: blue R, black I, grays B and BB, claret red, Bordeaux red, and brown B. The results secured have deviated in no respect from those obtained by E. V. Cowdry ('19), who first utilized the whole series of Janus dyes. Black I stains mitochondria, specific granules of islet cells, and nerve elements green, due presumably to the admixture of Janus green B with a second component. Janus blue R colors mitochondria much less intensely than Janus green B, but in addition stains the reticulum fibers—a phenomenon not obtained with either green B or black I. The other dyes of this series exhibited no specific staining affinities when applied to living islet cells.

3. *Thiazin dyes*

Thionin, methylene blue, and toluidene blue were applied in the same way as Janus green. They behave in much the same way as neutral red, but color also nerve fibers and, when too concentrated, the muscle fibrils of arterioles in addition.

In the acinar cells these dyes stain vacuoles of the same size and distribution as those which have an affinity for neutral red. Within the islet cells colored vacuoles are observed not only at the periphery, which is characteristic of the neutral-red reaction, but also larger ones deeper in the interior of the cells and sometimes in apposition to the nucleus. But they do not occupy the spaces of the canals of Holmgren.

4. Dahlia (Grübler & Co.)

Satisfactory results were not obtained with this dye (1:1000 to 1:10,000 by the drip method), because it exerted a marked toxic effect upon the cells. The mitochondria did not accept the dye, but the granules of the islet cells were sharply stained.

5. Bismarck brown (Vital, National Aniline and Chemical Co., lot no. 3972)

The nuclei and basal portions of the acinar cells were stained by a 0.01 per cent solution in normal saline injected intraperitoneally one-half to one hour previous to mounting the pancreas for observation. The mitochondria appeared faintly tinged with the dye in scattered acinar cells. The specific granules of islet cells stained when concentrations as low as 0.001 were applied.

6. Bindschendler's green (Grübler & Co.)

This dye is only notable because of its toxicity for both acinar and islet cells even when applied in concentrations as low as 1:10,000. The effect is more pronounced in islet than in acinar cells. Shortly after the application of the dye, the cytoplasm of the islet cells appeared crowded with watery vacuoles—an appearance simulating that described by Bensley as characteristic of isolated islet cells mounted in Ringer's fluid for a period of one-half hour. This toxicity is of interest because it may mean that the dye would be useful as a means of experimentally destroying islet tissue in still-living animals.

THE RESPONSE OF LIVING ISLET CELLS TO DEXTROSE, INSULIN, AND ALKALOIDS

1. Dextrose

In selecting possible substances the introduction of which into the body might be expected to accelerate the rate of insulin production, dextrose must be given first rank.

Accordingly, a large series of experiments was conducted to determine the effect of injections of dextrose in varying amounts upon the activity of islet cells. The dextrose was administered subcutaneously at intervals preceding anesthesia and after control observations fifteen minutes or more in duration. In all, fifty-seven negative and eight positive experiments were made upon mice in different stages of nutrition. The experiments were called negative when no change could be detected in the appearance of the living islet cells when carefully watched for a period of about two hours following the preliminary injection. The protocols of five of the eight positive experiments will be considered in detail.

a. Mouse no. 154; weight, 16.5 grams. Last feeding twenty-four hours previously. 10.35 A.M., received 0.05 cc. of 0.05 per cent atropine sulphate injected subcutaneously. At 10.50, also subcutaneously, 0.02 cc. of 2.5 per cent amytal, and at 10.58 A.M., 1 cc. of 20 per cent dextrose. At 11.22, the peritoneal cavity was opened, and shortly after, a large interstitial islet was selected for observation. During the next twenty-five minutes, a selected group of islet cells, under continual observation, showed no signs of activity, with the exception of the coalescence of two lipoid droplets located near the nucleus of one cell. At the end of this period, 11.47, a rush of fluid obscured the vision and the preparation was reset, another islet being obtained. A second 1 cc. of dextrose was injected at 11.52 A.M.

Within three minutes following this injection (11.55), a large watery vacuole was noted at the capillary extremity of one of the cells which was being studied. The vacuole was somewhat larger than the nucleus, which was situated at the other extremity of the cell. Undergoing constant changes in shape, it appeared to withdraw toward the interior of the cell, becoming progressively smaller as it did so. Twenty-seven minutes later (12.22), despite a third injection of dextrose at 12.15 P.M., its volume was reduced to one-third of its original size. At the end of an hour it had not completely disappeared. At 1 o'clock the circulation became quite sluggish and stopped. No other islet cells appeared to be active.

b. Mouse no. 167; 15 grams. Last feeding eight hours previously. Induction of ether anesthesia at 3.55 P.M. The islet, an interstitial one in close proximity to one of the large vascular channels, was placed under observation at 4.00. From 4.00 to 4.38, a control observation was conducted during which the islet cells showed no activity. At 4.38 P.M., 1 cc. of 20 per cent dextrose was injected subcutaneously. Ten minutes later, numerous large vacuoles appeared in the cells of this islet, whereas in the preceding protocol only a single cell showed this change. Two of these cells were selected for observation because their relation to the nucleus of the containing cell and to the cell membranes could easily be defined. The vacuole in the first cell at the beginning of examination rested against the nucleus and was larger than it in size. Within the space of twenty minutes, it had materially decreased in size, undergoing at the same time alterations in shape and slight changes in position. The cell containing this vacuole immediately overlay a capillary so that the vacuole was in particularly close contact with the blood stream. Observation of the second cell with its vacuole began at 5 P.M. At this time it was somewhat larger than the nucleus of the cell to which it belonged and was separated from the surface of a capillary by a thin fringe of cytoplasm. Within the space of the succeeding ten minutes, it became smaller and smaller, changing shape continually, and gradually withdrew toward the center of the cell. It is possible that this central withdrawal of the vacuole was occasioned only by its reduction in volume. At 5.16 P.M., the experiment was discontinued.

c. Mouse no. 175; 24 grams. Last feeding (bread, milk, and carrots) eight hours previously. At 3.20 P.M., 0.03 cc. of 5 per cent amytal was injected subcutaneously. Complete anesthesia did not follow and ten minutes later an additional 0.02 cc. was injected. At 3.40 P.M., the peritoneal cavity was opened and the pancreas mounted for observation, just as in the case of the other mice. At this time 1 cc. of 20 per cent dextrose was injected. One of two closely adjacent interstitial islets was selected and several ideally located cells were placed under observation. No changes were seen in them until 4.10, at which time the animal came out from the anesthesia. At this time another 0.01 cc. of amytal was injected and another islet, a small interlobular one, was studied. A second 1 cc. of dextrose was injected. Seven minutes after this injection, a large vacuole, which might have escaped detection previously because of its resemblance in size to a nucleus, was noted in close proximity to the nucleus of a cell. Near to this vacuole were two smaller ones which were shortly incorporated into it. In closely adjacent cells other large vacuoles were noted, but their changes were not followed. At 4.45 the camera lucida was adjusted and a drawing made of the cell containing the vacuole mentioned. At short intervals other drawings were made indicating the relation of the vacuole to the cell and its changes in shape and position. By reference to figure 4 it will be seen that within the twenty-one minutes following the first drawing, the vacuole moved out over an underlying capillary, distending the cell membrane in its course. During the next fifteen minutes its position in the cell was changed, but not its size. In the succeeding hour the vacuole underwent a marked reduction in size, as is obvious from a comparison of stages 11 and 12, figure 4. At 7.05, the vacuole had withdrawn to the center of the cell and was so small that it was outlined with difficulty at a magnification of 625 diameters. Circulation became quite sluggish at 7.30 and the experiment was stopped.

d. Mouse no. 202; 13.5 grams. Last feeding fifty-six hours before. At 1.30 P.M., it received subcutaneously 0.02 cc. of 2.5 per cent amytal. Ether anesthesia was commenced immediately following the injection. The pancreas was mounted for observation and at 1.41 a small interstitial islet was selected for the experiment. No activity was manifest during a control observation of ten minutes. At 1.50, 1 cc. of 20 per cent dextrose was injected subcutaneously, but the islet was lost, due to movement of the animal during the injection. Another small interstitial islet was immediately found. At

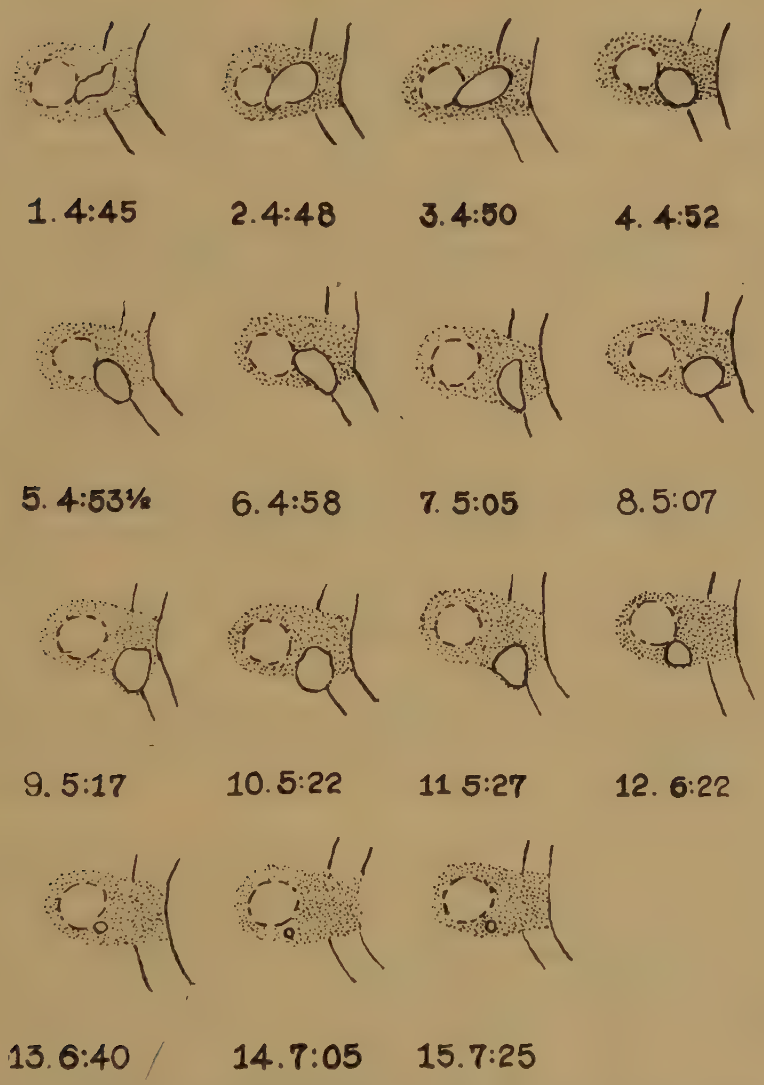
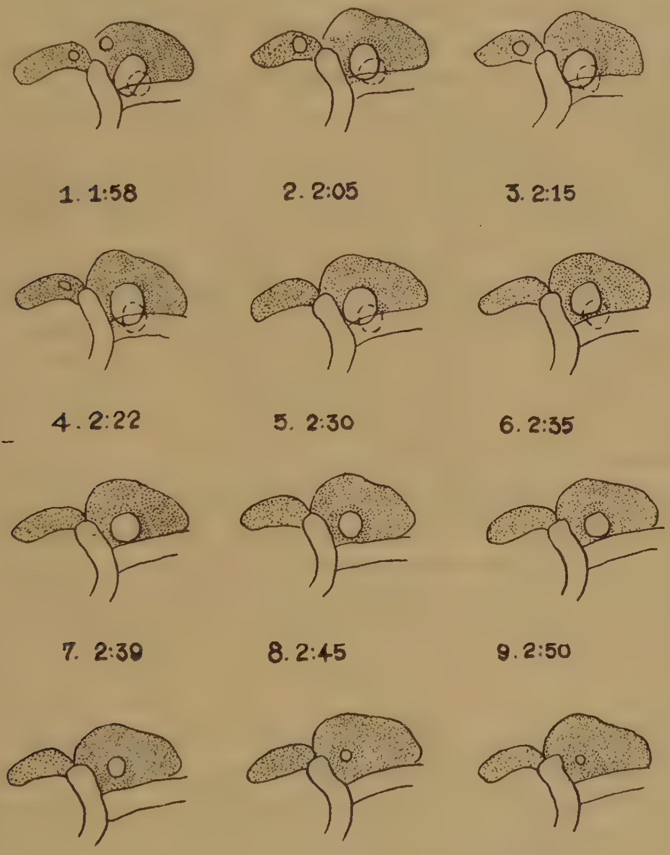

1. 4:45
2. 4:48
3. 4:50
4. 4:52
5. 4:53½
6. 4:58
7. 5:05
8. 5:07
9. 5:17
10. 5:22
11 5:27
12. 6:22
13. 6:40
14. 7:05
15. 7:25

Fig. 4 Mouse 202. Reaction of living islet cells to the introduction of dextrose. A series of camera-lucida drawings of the same islet cell, showing stages in the migration of a large vacuole, which formed in the cytoplasm twenty minutes after the subcutaneous injection of 1 cc. of 20 per cent dextrose, and its gradual reduction in size. The vacuole is heavily outlined, the nucleus indicated by broken line, the cytoplasm stippled, the capillary margins are indicated by heavy lines. Amytal and ether anesthesia. Zeiss apo. 2 mm., 1.3 n.a. × 625.

1.54 a large vacuole was observed in a cell located at its periphery. The intercellular clefts separating this from neighboring cells were not clear, but it was discovered that the nucleus of the cell containing the vacuole lay immediately beneath the vacuole. In a neighboring cell was a smaller vacuole which soon disappeared. At 1.58 the camera lucida was adjusted and the first drawing made (fig. 5). This indicates the relation of the large and small vacuoles already mentioned to the contiguous capillary and also the position of a third small vacuole which made its appearance between 1.55 and 1.58. The first of the smaller vacuoles disappeared at 2.01 and the second at 2.35. The remaining camera-lucida drawings illustrate the gradual decrease in volume of the larger vacuole. It became too small for accurate camera-lucida drawings at 3.15, and at 3.25 it likewise had completely disappeared. Throughout the experiment, blood circulated at the usual rate, except for a period of two minutes between 2.37 and 2.39 and of one minute between 2.42 and 2.43, when it slowed down. During the entire period of observation, no movement of specific granules or other formed materials was noted in the cells included within the field of observation, nor did any other cells of this islet show the formation of vacuoles. Anesthesia was light throughout the study. The experiment was terminated at 3.30.

e. Mouse no. 219; 25.3 grams. Last feeding twenty-four hours previously. Injected 0.2 cc. of 12 per cent urethane at 9.30 A.M. At 9.38, an interstitial islet was located. During a control observation of twenty minutes, the cells in focus showed no signs of activity. At 9.58, 0.4 cc. of 20 per cent dextrose was injected subcutaneously. During the ensuing forty-five minutes the cells remained apparently quiescent. At 10.45, the preparation was readjusted and another small islet brought into focus. At 10.53, two vacuoles were observed in the peripheral cytoplasm of a single cell. One of these underwent rapid diminution in size and had disappeared completely by 11.00; the other increased in size, elongated, and then became elbow-shaped. By 11.10 it had migrated to the capillary surface of the cell, where it soon assumed a multilocular shape. Beginning at 11.15, it decreased in size rapidly and had completely disappeared at 11.25. Vacuoles were observed in no other cells of this islet nor in the surrounding acinar tissue. The flow of blood was rapid throughout the course of the experiment.



1. 1:58 2. 2:05 3. 2:15

4. 2:22 5. 2:30 6. 2:35

7. 2:39 8. 2:45 9. 2:50

10. 3:02 11. 3:07 12. 3:12

Fig. 5 A series of camera-lucida drawings, illustrating, like figure 4, the secretory response of islet cells to the introduction of dextrose. The first drawing was made eight minutes following the subcutaneous administration of 1 cc. of 20 per cent dextrose. The capillary margins are outlined in black, the cytoplasm of the cells stippled, secretory vacuoles heavily outlined. The nucleus of but one cell was apparent at this focus. It is indicated by a broken line. Zeiss apo. 2 mm., 1.3 n.a. × 625.

The incidence of successful results seems at first to be surprisingly low, but several non-controllable factors besides the necessity for anesthesia enter into each experiment. Inability to distinguish the 'a' from the 'b' variety of islet cells in the living unstained state has made it a matter of conjecture in each case as to whether or not 'b' cells were under observation, since the 'a' variety, according to the evidence of Homans, is apparently not responsible for the secretion of insulin. Further, the act of production of insulin is probably shared by the cells in a very large number of islets. Bensley has estimated an average total of about 30,000 islets for the pancreas of the guinea-pig; and personal counts by his methods of the islets in the pancreases of six mice of the size and strain used in the experiments have shown from 450 to 700 of them to be present. There is not much choice in the selection of islets, for in twenty-seven of the mice studied only one islet was bared for observation in the portion of the pancreas available for examination. In thirty others an alternative between one of two islets was possible. In the remainder of the experiments three to five islets were observed in one preparation. Unless, therefore, it is assumed that all the islet cells throughout the pancreas are working simultaneously and to the same extent, the possibility is evident that some will respond less readily to dextrose than others.

2. *Insulin*

Twenty-six mice were utilized to test the effect of insulin upon apparently quiescent islet cells. Twelve of these mice were anesthetized with amytal, and after an islet had been selected and observed for twenty or thirty minutes, subcutaneous injections of 0.05 to 0.1 cc. of Squibb insulin (10 units per cubic centimeter) were made every five minutes for from one to two hours. No alterations in the appearance of the cells, dimensions of the capillaries, or rate of blood flow through the islet were noted in any of the mice of this series.

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Since the action of insulin depends upon the metabolic rate of the animal, its effectiveness would be decreased under amytal narcosis (Macleod, '24, and Britton, '25). Accordingly, two other experiments were conducted in which the mice were not anesthetized until the effect of the insulin had been realized. Seven mice received sufficient insulin to produce a typical reaction, then a minute dose of amytal was injected to insure that the animal would remain quiet throughout the study. The peritoneal cavity was opened, the pancreas quickly mounted, and an islet selected for observation. The remaining seven mice received daily injections of 0.05 cc. of Squibb insulin until they finally responded to this small dose with a marked coma. Then, during coma, the pancreas was mounted and an islet selected for study as in the preceding experiment. In neither case did the islet cells differ from the quiescent state nor was the width of the islet capillaries altered.

3. *Alkaloids*

a. Pilocarpine. Whether pilocarpine accelerates or diminishes the activity of islet cells depends upon whether there is a predominance of acceleration or inhibition by the vagus nerve. Clark ('25) has demonstrated that drugs which stimulate the parasympathetic lower the blood sugar, provided that sympathetic glycogenesis is prevented by the simultaneous administration of ergotoxin. If the right vagus is sectioned, the hypoglycemia does not ensue. Britton ('25) has likewise lowered the blood sugar by electrical stimulation of the vagus on the cardia. Precautions were taken to exclude the possibility of exhaustion of glycogen stores in the liver.

Pilocarpine in varying amounts from 0.01 cc. of 0.01 per cent to 0.1 of 0.1 per cent was injected subcutaneously into a series of twenty mice at short intervals preceding and during the study of the islets. These experiments gave uniformly negative results, because no specific granules were eliminated nor were the vacuoles which appear in response to the introduction of glucose observed in any case, even though the observations were continued up to three hours following the initial introduction of the drug.

These negative results are not, however, to be considered as final, despite the fact that surrounding acinar cells in thirteen experiments gave the typical secretory response to pilocarpine described by Covell ('28); for in several guinea-pigs which were injected with minute doses of pilocarpine and after a lapse of one-half hour were killed and injected through the aorta with 1:10,000 Janus green B (Bensley's method), vacuoles were seen in the cytoplasm of islet cells which were in every respect similar to those whose development and fate were followed in mice presumably responding to dextrose injections. Further experiments to determine the effect of pilocarpine upon the islet cells are under way and will be reported subsequently.

Certain vascular phenomena which may be significant were detected in the islets of mice which were given lethal doses of pilocarpine. If the circulation was sluggish at the beginning of observation, as frequently happened in urethanized mice, the rapidity of flow was markedly increased shortly after the administration of the drug; while in occasional capillaries, the plasma relative to the cell volume of the circulating fluid was increased. Immediately preceding death and coincident with labored breathing, the flow in the islet capillaries oscillated: corpuscles which were observed to be moving rapidly in the arteriovenous direction stopped suddenly and hurried back in the opposite direction, only to have their direction again reversed and flow toward the veins. This type of oscillation might occur for several minutes preceding death.

b. Atropine sulphate. Five experiments were conducted to determine the effect, if any, of atropine sulphate upon islet activity. The results were uniformly negative. A typical protocol (mouse no. 146, female, weighing 17.5 grams) records the injection of 1.25 cc. of 0.05 per cent atropine sulphate in quantities varying from 0.005 cc. at the beginning of observation to 0.5 cc. two hours later. At no time were specific granules observed to leave the group of cells under observation nor did the formation of intracellular vacuoles follow the introduction of the drug.

DISCUSSION

The relation of the canalicular apparatus of the islet cell to the vacuome as described by Parat ('28), Guilliermond ('27), and others in a variety of animal and plant cells must necessarily await further advances in microchemical methods as applied to living animal cells. Covell and Scott ('28) and Cowdry and Scott ('28) have initiated progress in this direction by showing that the vitally stained vacuome of nerve cells and of the malarial parasite blackens with osmic acid. All transitions have been described between aligned neutral-red vacuoles in living cells and the complete osmicated Golgi network. In these researches is found a wholly new line of evidence supporting the contentions of Parat.

Owens and Bensley ('29) have recently commented upon one of the discrepancies which exist between Bensley's observations upon the vacuome of islet cells and those supplied by the other adherents of the vacuome theory, namely, the appearance of the islet-cell vacuome as a closed system of canals rather than as discrete vacuoles. They regard such a difference as of minor importance as compared with agreement upon the major treatise that the reticular apparatus of Golgi is the fixed tissue equivalent of the vacuolar apparatus of animal and plant cells. In so far as the form of the apparatus is concerned, the observations detailed in this paper offer ample confirmation of Bensley's description in cells under ideal conditions for prolonged observation; and the fact that changes from the vacuolar to the canalicular form and vice versa have not been observed during numerous studies of two or more hours' duration is not contradictory to his theory that changes from one form to the other are explainable in terms of surface-tension phenomena. In favor of Bensley's theory is the occurrence in a few cells of material optically similar to the canals of Holmgren, but arranged in discrete vacuoles. Another difference which remains to be explained is that the islet-cell vacuome does not segregate neutral red.

It has not been possible to demonstrate the canalicular apparatus in cells which are in an active stage of secretion. Whether this difficulty is due to its dissolution during the secretory process or to an actual compression of the apparatus into smaller compass due to the large vacuoles suddenly formed within the cytoplasm is a moot question.

It has been demonstrated that although the specific granules are not thrown into the blood stream in response to the introduction of dextrose, the islet cells do not remain inactive. Large vacuoles, 100 cu.μ or more in volume, have been observed to form in that portion of the islet cell which experience has taught is most likely to lodge the canalicular apparatus. Rarely does more than one vacuole form in the cytoplasm of a single cell and usually only a few cells of an islet are active in this way, the others remaining apparently quiescent throughout experiments of several hours' duration.

The formation of these vacuoles in the islet cells of mice presumably responding to an overbalanced carbohydrate metabolism is most simply explained as a change in the hydrophilic capacity of the cell colloids intimately related to secretion. Whether the specific granules become incorporated in the fluid of vacuoles or the vacuoles are formed by liquefaction of the granules is not possible to determine by means of the best optical equipment available. However this may be, the vacuoles move toward the capillary end of the cell, where they decrease in size, their contents being presumably passed by diffusion through the cell membrane into the blood stream.

Though the evidence seems strong that these vacuoles are secretory in nature, there are other possibilities. They may be the products of cytoplasmic degeneration resulting from the trauma of manipulation, for vacuolar degeneration does occur in islet and acinar cells subjected to trauma. However, in the islet cells the vacuoles in vacuolar degeneration are minute and are scattered evenly through the cytoplasm, as Bensley ('11) has shown in an examination of postmortem changes in isolated cells mounted for a half-hour or more in Ringer's solution. Then again, cells undergoing vacuolar degeneration show characteristic nuclear changes and rapid disintegration of the peripheral cytoplasm which were not observed in the islet cells described in these experiments. It is unlikely that the vacuoles represent a normal excretory mechanism by which the cells rid themselves of waste materials, because, if so, one would expect them to appear in control mice held under observation for the same periods of time, but not under the influence of injected dextrose.

The conception that the secretory products of endocrine cells are converted into soluble form for extracellular transport is not a new one. Homans, fifteen years ago, suggested the possibility that the specific granules of the islet cells are thus changed into an intermediate state prior to secretion. Bensley ('15), studying the opossum thyroid, obtained abundant evidence that the secretion destined for the blood stream is in the form of vacuolar material which accumulates at the poles of the cells next the capillaries.

The elimination of specific granules directly into the blood stream without the intervention of a fluid state demands necessarily a mechanism for the propulsion of granules from a cytoplasm of but slightly less viscosity through the cell membrane and into the blood stream. These experiments revealed no mechanism capable of performing such a task.

What changes, if any, in the islet cells might be expected to follow the administration of insulin is a matter for conjecture. The introduction of the hormone did not initiate a secretory response in quiescent cells. But retention could well result following a sudden influx of insulin. No experiments have been conducted as yet to determine the effect of insulin upon cells in active secretion.

Pilocarpine has not been shown to influence the formation of secretory vacuoles within islet cells even in concentrations effecting active secretion in acini adjoining the islet under observation. This lack of success may be attributed to a differential susceptibility in the response of nerve endings in acinar and islet tissue, those of islets succumbing to the toxic effects of doses which produce active acinar secretion. That atropine fails to inhibit the secretory response of islet cells under the influence of dextrose is confirmatory evidence that secretion may proceed in the absence of vagus innervation.

CONCLUSIONS

1. These observations on living islet cells of mice with their circulation intact support Bensley's ('11) description of the canalicular apparatus in fresh isolated cells of the guinea-pig.
2. The canalicular system of the islet cells does not segregate the dye neutral red when it is applied vitally by appropriate methods.
3. The mitochondria of living islet and acinar cells may be successfully stained with Janus green B.
4. In response to the introduction of dextrose, large vacuoles are observed to form in that region of the islet cell lodging the canalicular apparatus and migrate to the periphery of the cells next the capillary, where they are diminished in volume, presumably by diffusion of their contents through the cell membrane. This phenomenon is never observed during control observation and is thought to constitute the mechanism of insulin secretion.
5. Specific granules or other cytoplasmic inclusions are not removed from islet cells following the introduction of dextrose.
6. Pilocarpine has not been successfully used to influence the formation of secretory vacuoles in islet cells.

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BONE-MARROW REACTIONS

II. THE BLOOD COUNT IN THE ALBINO RAT: BLOOD PLATELETS

FRANCIS D. GUNN AND STUART L. VAUGHAN
Department of Pathology, Northwestern University Medical School, and Wesley Memorial Hospital

METHOD

That there is no entirely satisfactory method for the enumeration of blood platelets is evident from the number of new methods or modifications of old methods appearing every year in the literature. Methods that are suitable for clinical work are frequently not applicable to experimental work, especially on small animals, either because they require too large amounts of blood or because they are not sufficiently accurate. Various modifications of the direct method devised by Oluf Thomsen ('20) seem to have given the most consistently reliable results in different hands. In the experience of the writers, the modification described by Reimann ('24) is the most satisfactory for counts in the human and in laboratory animals where venepuncture can easily be executed. The impracticability of this method in experiments on rats where repeated heart puncture would have to be performed is obvious.

Consequently, an indirect method was used, similar to that described by Gunn ('26) for platelet counts in rabbits. It is essentially that use by Cramer, Drew, and Mottram ('23), Bedson and Zilva ('23), Sooy and Laurens ('24), and others, with the difference that instead of counting the wet preparation under a cover-slip, we make the counts on dried, stained films similar to the technique of Fonio ('12).

Technique

The diluting fluid used was isotonic (3.8 per cent) sodium citrate with the addition of 0.5 per cent neutralized commercial formalin. (The sodium chloride used in 1926 (Gunn, '26) has been found to be superfluous.)

The fluid is drawn into a leucocyte diluting pipette until the bulb is half filled and then emptied into a shallow depression in a clean paraffin block. The tail is prepared and clipped off as described in the previous paper and immediately dipped into the diluting fluid, one large drop being allowed to flow into the fluid. (A second dilution may be prepared by quickly transferring the end of the tail to a second depression in the paraffin block containing a similar quantity of fluid, and used as a check on the first.) This mixture is then drawn into the pipette previously wet with the fluid and followed by enough diluting fluid to fill the bulb. After shaking for two or three minutes, the first few drops are discarded and thin films are made on scrupulously clean slides. The firms are dried, stained with Wright's blood stain, covered with oil and large cover-slips, and counts made under the 4-mm. objective and 10× ocular of a binocular microscope. To aid in the counting, an ocular net (ruled glass disc which lies on the diaphragm of one ocular) was used.

In these preparations the erythrocytes appear bluish and the platelets, brick red or purplish red. When platelets lie above or below the erythrocytes, the latter are sufficiently transparent so that the platelets, much more heavily stained, are clearly visible through them. If the films are allowed to dry for at least fifteen minutes in the air, the cells do not wash off the slide. When the latter does occur, it is due to imperfect cleaning of the slide and round, clear spots on the film indicate the places where cells have washed away.

The chief advantages of this method are: first, that the platelets are clearly stained and cannot be confused with dust particles, hemoconia, precipitated stain, or other extraneous material and, secondly, that the slides are permanent and the counts can be made at any convenient time. A homogeneous mixture of cells is insured by thorough agitation of the diluted blood in the pipette. The chief source of error is that inherent in every other method for platelet enumeration, namely, clumping of platelets at the time the blood is taken due to contact of the blood with tissue juice or foreign objects to which they adhere. This danger has been reduced to a minimum by allowing the blood to flow from the severed vessels directly into the anticoagulant fluid, and rarely has it been necessary to discard a slide on account of the appearance of clumping.

When the blood was taken from the femoral vein, the animal was prepared as described in the previous paper (erythrocytes and hemoglobin), and after exposing the vein, three or four drops of the diluting fluid were dropped into the site and the vein snipped through the fluid so that the blood mixed with the anticoagulant as it flowed from the vein. This mixture was taken up immediately into a pipette, shaken thoroughly, and films prepared in the usual way. This technique was not so satisfactory as the tail method, chiefly because of the greater danger of admixture with tissue juice and because of the difficulty of controlling the flow of blood. Consequently, certain discrepancies occurred in the first group that did not appear in the second (table 1).

In determining the erythrocyte-platelet ratio, about 2000 erythrocytes were counted (1000 on each of two slides) for each estimation and the results expressed in terms of platelets per one hundred erythrocytes (table 2). In a film of average thickness about twenty visual fields will give this number.

It has been thought by workers who accept Wright's theory of the origin of platelets that the elongate forms, those with long processes, and especially the slender, spindle-shaped platelets represent a young or immature form and that their appearance in the blood stream is due to rapid production, with irregular pinching off of the processes of the megakaryocytes. If this is true, we might expect a larger proportion

TABLE 1

Platelets in thousands; counts at one-week intervals

Group I

Rat								
C3	781	1388						
C8	1300	1005	1190					
C5	966	1213	1406	1530				
C7	1239	1012	1222	1361	1366			
C2	....	1589	1517	....	1212	1605		
C4	1202	1711	1468	1509	1300	1469	1264	
C6	591	1349	1565	1454	1383	1352	1264	1261
Average,	1013	1324	1395	1464	1315	1475	1264	1261

Group II

Rat								
C15	1372	1514						
C14	1431	1509	1732					
C10	1418	1422	1430	1469				
C11	1269	1812	1544	1223	....			
C9	1154	1213	1561	1538	1425	1487		
C13	1257	1185	1507	1793	1470	1547	1354	
C12	1027	1019	1349	1213	998	1490	....	1558
Average,	1275	1382	1521	1447	1298	1508	1354	1558

TABLE 2

Platelets per one hundred erythrocytes

Group I

Rat								
C3	8.9	17.1						
C8	12.9	10.9	12.7					
C5	8.4	13.4	15.8	16.8				
C7	11.9	13.6	14.0	14.3	14.5			
C2	...	17.0	16.6	...	13.2	16.1		
C4	10.8	19.1	16.7	16.1	13.0	15.6	14.3	
C6	7.0	16.7	17.8	15.6	14.9	14.6	14.0	14.9
Average,	10.0	15.4	15.6	15.7	13.9	15.4	14.2	14.9

Group II

Rat								
C15	13.6	15.4						
C14	15.4	16.0	17.8					
C10	14.5	13.7	14.9	14.4				
C11	13.5	19.5	17.1	14.3	...			
C9	12.1	12.8	17.9	17.7	16.7	18.1		
C13	12.4	12.8	17.4	25.0	15.6	16.8	14.7	
C12	11.2	10.7	15.4	14.1	12.5	16.2	...	17.5
Average,	13.2	14.1	16.8	17.1	14.9	17.0	14.7	17.5

of these forms in conditions favoring rapid increase in their numbers. Experimental support for this idea has been furnished by results obtained in rabbits exhibiting increase in blood platelets due to bacterial infection (Gunn, '27). In those experiments the spindle forms were increased from a level of 2.5 to 8.5 per cent before injection to a maximum of about 10 to 15 per cent after injection. Consequently, in our estimations, in the majority of cases we counted the slender, spindle forms and expressed their number in terms of per cent of the total platelet count. The results on these two groups of 'normal' rats have been tabulated for comparison in table 3. The normal values lie between 2.3 and 10.8 per cent (average, 5.6 in group I and 6.1 in group II).

TABLE 3

Platelet spindle forms in per cent

Group I

Rat								
C3	2.8	3.5						
C8	2.3	3.9	5.7					
C5	3.3	2.7	5.5	5.1				
C7	5.6	...	4.8	6.2	3.1			
C2	...	4.7	6.3	...	3.9	5.8		
C4	5.9	5.6	5.2	4.3	4.1	6.3	3.1	
C6	...	...	6.5	10.8	8.9	5.2	5.8	9.2
Average,	4.0	4.1	5.7	6.6	5.0	5.8	4.5	9.2

Group average, 5.6.

Group II

Rat								
C15	8.4	7.0						
C14	3.0	4.0	5.1					
C10	4.3	7.0	5.6	3.3				
C11	4.3	5.9	7.4	4.8	...			
C9	4.8	5.4	7.1	6.3	6.9	4.8		
C13	6.8	3.0	4.8	5.1	7.8	8.8	8.9	
C12	6.2	7.1	5.2	3.7	7.0	3.7	...	5.7
Average,	5.4	5.6	5.9	4.6	7.2	5.8	8.9	5.7

Group average, 6.1.

In order to determine the probable error in the method, five separate determinations on the same animal were made on blood from the tail, one after the other as rapidly as possible, and the results tabulated (table 4). The erythrocyte count was 9.7 million. The maximum variation from the mean in these counts is 128,000, or about 8.7 per cent. The possible error in the estimation of spindle forms is necessarily greater, because the count is based upon a smaller total number (about 300 to 350) of cells (platelets). These counts were made with usual care, and we consider that in our hands the error in technique is within 10 per cent. Needless to say, this does not include the error in estimating the absolute number of erythrocytes, which must be taken into consideration in any indirect method.

TABLE 4

Repeated counts on the same rat; dry-film method

Erythrocytes counted	*Platelets per 100*	*Platelet-erythrocyte ratio*	*Platelets in thousands*	*Spindle forms, per cent*
2105	15.1	1–6.6	1430	9.5
2241	16.8	1–5.9	1591	9.3
2313	15.1	1–6.6	1430	7.5
2064	14.1	1–7.1	1335	9.3
2228	15.8	1–6.3	1496	8.3
Average,	15.38	1–6.5	1456	8.78

Another question which arose in connection with the use of this method was whether or not the results so obtained were similar to those obtained by the more commonly used method of counting in wet-film preparations. To answer this, we have made counts on the same animal at the same time by both methods, each technique executed in duplicate. The diluting fluid used for the wet preparations was that of Rees and Ecker (sodium citrate, 3.8 per cent; commercial formalin, 0.2 per cent; brilliant cresyl blue, 0.1 per cent). The dilutions were made in the usual manner, but instead of preparing dry films, we placed a standard drop of the mixture on a slide, covered with a thin cover-slip, sealed with melted paraffin, and made the count as usual. One of these preparations was counted under the oil-immersion objective (Koristka 2 mm., N.A. 1.30) and the other under the 4-mm. dry lens. Matched compensating 10× oculars and achromatic condenser N.A. 130 were used throughout.

The figures tabulated in table 5 show the results of this procedure. All four counts are well within the limits of experimental error, and we may conclude that in our hands the results obtained by the two methods are practically identical.

TABLE 5

Comparative counts on the same rat; dry- and wet-film methods

Erythrocytes counted	*Platelets per 100*	*Platelets in thousands*	*Method used*	*Microscope objective*
2268	14.9	1342	Wet film	Oil immersion, 2 mm.
2056	14.5	1306	Wet film	Dry lens, 4 mm.
2145	14.7	1324	Dry-stained	Dry lens, 4 mm.
2143	14.1	1270	Dry-stained	Dry lens, 4 mm.

COMMENT

As will be seen by reference to the tables, there is a distinct tendency toward elevation of the platelet number after the initial count. This is true both of the percentage figure and of the calculated number and occurs at the time the erythrocytes are showing the greatest diminution, clearly demonstrating that this rise is not dependent upon fluctuations in the red-cell number. Whether this change is due to the anesthetic, to the loss of blood, or to both is of little importance for the present purposes, as these factors will be practically constant in all future experimental procedures.

In spite of a few apparent discrepancies in individual counts in group I (initial counts in C3, C5, and C6), the averages by weeks in the two groups are fairly close to each other, the counts in the second group being somewhat higher. The averages of the thirty-three counts in each group are 1,314,000 in the first and 1,418,000 in the second—a difference of slightly less than 8 per cent. In the second group the maximum variations in individual counts are found in rats C11 and C13, the greatest variation from the initial count in each of these amounting to about 43 per cent. Rat C13, as was mentioned in the previous paper, had a subcutaneous abscess which was noticed at the time the third count was made and which apparently had healed by the time the fourth count was made. Rat C11 appeared perfectly well and showed no evidence of infection throughout the period.

Comparing these figures with those obtained by others on normal rats, we find that the platelet percentage and the calculated platelet number are considerable higher than those found by other workers, except Bedson and Zilva ('23). These authors, using blood from the tail and the indirect method of counting, obtained in seven normal, young rats an average of 1,208,857 platelets and 7.8 million erythrocytes; ratio about 1:6.5. The exact age of their animals was not given, but they were much smaller than those used by us (about 50 grams). Taylor ('15), the first to publish results of platelet counts in rats, obtained in eighteen healthy albino rats an average of 1 million platelets and 10 million red cells. Cramer, Drew, and Mottram ('23) found about 800,000 with 9 to 10 million red cells in animals weighing from 50 to 150 grams. Laurens and Sooy ('24), in six-month-old rats kept in room light, found by the indirect technique 762,000 platelets and 7.3 million erythrocytes (ratio, 1:10). Stammers ('25) states that in his colony of normal rats the level is slightly under 900,000. Direct methods in which measured dilutions are made in an erythrocyte pipette usually yield much lower results (Shulman and Mendel, '24; Laurens and Sooy, '24). The results obtained by us in group II, the animals in which we used the tail blood for counts, are practically identical with those of Bedson and Zilva, the average in the initial counts being 1,275,000 platelets with 9.6 million erythrocytes; ratio, 1:7.5. It seems probable that the differences between our results and those of most other workers are to be explained by differences in the strains of rats used, and not to differences in technique, since we have shown that the different methods commonly employed are capable of giving identical results.

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BONE-MARROW REACTIONS

III. THE BLOOD COUNT IN THE ALBINO RAT: LEUCOCYTES, WITH SPECIAL REFERENCE TO THE ARNETH COUNT

STUART L. VAUGHAN AND FRANCIS D. GUNN
Department of Pathology, Northwestern University Medical School, and Wesley Memorial Hospital, Chicago

ONE FIGURE

This paper is part of a study of the normal blood count in the albino rat. The results of the erythrocyte, hemoglobin, and platelet determinations are given in sections I and II.

METHODS

The leucocyte count was made as stated in section I. The films for the differential and Arneth counts were made on clean glass slides and stained by Wright's method. Care has been taken to spread the blood thinly and evenly, with drying hastened by an air current to prevent crenation of red cells and shrinking of leucocytes. The counts were made with an oil-immersion lens, the slide being moved transversely at intervals, never in the long dimension of the film, and all cells tabulated until one hundred polymorphonuclear cells had been encountered and classified according to Arneth's criteria. The differential count was made at the same time, and the results are, therefore, based upon an average of over 500 cells. The smallest number in any single count was 228. After the completion of these counts, the slides were reexamined and the polymorphonuclear cells classified according to the method of Cooke.

RESULTS

The leucocyte counts are given in table 1. They ranged in the initial counts in the two groups from 12,000 to 24,000 and from 10,000 to 27,900, respectively. In the subsequent counts there was considerable variation in both groups, the range in all counts being from 8300 to 27,900. The group averages were 15,900 and 13,300. In calculating the leucocyte averages the secondary counts of rat C13 were omitted, since a subcutaneous abscess developed about the third week.

TABLE 1

The leucocyte count

Group I		*Group II*	
RAT	THOUSAND	RAT	THOUSAND
C3	18.0 17.1	C15	14.2 11.5
C8	18.4 11.7 15.5	C14	14.3 16.9 12.3
C5	12.6 12.4 14.0 16.1	C10	27.9 16.2 15.7 16.4
C7	12.0 12.5 10.1 15.6 12.0	C11	12.5 12.0 16.4 12.6 11.2
C2	24.4 17.7 14.9 11.9 26.1 20.8	C9	12.3 11.8 8.5 8.3 9.9 8.8
C4	22.3 13.0 15.7 14.6 16.3 21.4 21.6	C13	13.1 15.4 16.0 11.8 13.8 14.7 26.2
C6	12.7 13.3 13.7 10.9 19.4 19.8 17.6 12.2	C12	10.8 10.2 13.4 9.9 10.8 12.3 8.4 10.0
Initial count average,	17.2	Initial count average,	15.0
Group average,	15.9	Group average,	12.6

TABLE 2

The leucocyte counts of other workers

AUTHOR	SOURCE OF BLOOD	AGE OF RAT	AVERAGE COUNT
Jolly	Peripheral	365 days	10,000
Rivas	Tail	Adult	9,560
Nowrey	Tail	163 days	9,280
Margot	Tail	148 days	9,370
Hammett	Tail	150–175 grams	9,740
Kleineberger and Carl	Thigh	Adult	15,200
Happ	Thigh	4 months	9,600
Happ	Thigh	9 months	8,000
Laurens and Sooy	Tail	6 months	9,060
Cannon		12–18 months	11,700
Taylor		Adult	19,000

Most other workers have obtained lower counts than those for this series. The results of Kleineberger and Carl ('12) were quite similar to ours, and Hammett et al. ('22) obtained practically as great variation in counts. The values published by others for adult rats are given in table 2. Our results are not in agreement with the observation of Happ ('22) that blood from the tail gives lower total leucocyte counts than does blood from the larger veins.

The differential count

The polymorphonuclear cells (table 3), including neutrophils, eosinophils, and basophils, in the initial counts of the first group varied from 11.9 to 23.7 per cent (average, 17.5 per cent). For all counts in this group the variation was from 11.9 to 28.6 per cent (average, 18.8 per cent). The range in the initial count for the second group was 11.8 to 28.1 per cent (average, 20.6 per cent).

TABLE 3

The polymorphonuclear count

Group I									*Group II*								
RAT	PER CENT								RAT	PER CENT							
C3	22.3	26.3							C15	19.6	9.9						
C8	23.7	20.4	24.3						C14	11.8	11.0	11.5					
C5	14.8	21.7	28.6	22.9					C10	24.1	17.3	19.4	12.8				
C7	16.4	24.2	25.0	25.7	24.0				C11	24.6	22.7	28.6	22.8	24.0			
C2	16.0	17.2	16.5	22.8	15.9	12.8			C9	28.1	22.2	25.5	20.7	23.2	21.9		
C4	11.9	16.6	15.4	13.8	15.0	20.5	16.6		C13	18.7	27.2	23.4	22.2	17.1	26.6	43.9	
C6	17.7	19.4	14.3	12.3	15.0	12.0	14.5	13.3	C12	25.0	27.0	16.8	19.6	28.9	19.5	17.9	23.6
Initial count average, 17.5									Initial count average, 21.7								
Group average, 18.8									Group average, 20.6								

Table 4 shows the eosinophil percentage. The range in the two groups was from 0.6 to 8.4 per cent (average, 2.6 per cent) and from 0.2 to 5.8 per cent (average, 2.4 per cent).

The basophil cells (table 5) ranged in the two groups from 0 to 0.9 per cent (average, 0.2 per cent) and from 0 to 1.3 per cent (average, 0.25 per cent).

The total initial lymphocyte count in the two groups (table 6) ranged from 75.6 to 86.9 per cent (average, 81.4 per cent) and from 65.4 to 87.7 per cent (average, 74.6 per cent). All counts in the first group varied within the limits of 69.7 and 86.9, with an average of 80 per cent, and in the second group between 48.7 and 88.2 (average, 76.9 per cent). It will be noted that there is considerable uniformity in the counts in both groups, with the exception of rat C13, whose lympho-

TABLE 4

The eosinophil count

Group I		*Group II*	
RAT	PER CENT	RAT	PER CENT
C3	2.3 2.1	C15	5.7 3.4
C8	1.7 3.9 0.9	C14	2.6 0.7 0.2
C5	3.3 2.8 6.3 2.8	C10	1.7 2.6 4.3 2.6
C7	2.5 6.5 5.3 8.4 6.0	C11	3.4 1.6 1.4 2.7 0.5
C2	2.0 1.7 1.7 2.5 3.0 2.7	C9	2.8 1.8 3.6 2.3 0.9 1.3
C4	1.8 0.8 3.1 1.5 2.8 1.2 1.3	C13	5.8 5.4 1.4 0.7 5.5 2.9 1.3
C6	0.7 1.0 0.6 0.8 1.8 2.0 1.5 2.8	C12	1.3 4.6 4.4 0.4 0.6 0.6 0.9 1.2
Group average, 2.6		Group average, 2.4	

TABLE 5

The basophil count

Group I		*Group II*	
RAT	PER CENT	RAT	PER CENT
C3	0.0 0.0	C15	0.2 0.1
C8	0.7 0.2 0.2	C14	0.1 0.0 0.2
C5	0.0 0.9 0.6 0.0	C10	0.2 0.6 0.1 0.0
C7	0.0 0.7 0.0 0.0 0.0	C11	0.2 0.0 0.6 0.0 0.5
C2	0.0 0.2 0.7 0.0 0.2 0.4	C9	0.0 0.2 0.0 0.0 0.0 0.0
C4	0.0 0.0 0.5 0.1 0.0 0.0 0.7	C13	0.2 0.8 0.2 0.7 0.0 0.3 0.4
C6	0.2 0.2 0.1 0.2 0.0 0.0 0.2 0.3	C12	1.3 0.6 0.3 0.2 0.3 0.0 0.0 0.5
Group average, 0.2		Group average, 0.25	

TABLE 6

The total lymphocyte percentage

Group I		*Group II*	
RAT	PER CENT	RAT	PER CENT
C3	76.6 71.1	C15	78.3 86.3
C8	75.6 78.6 73.8	C14	87.7 88.2 86.8
C5	83.8 78.3 71.5 74.8	C10	73.0 79.1 79.4 85.0
C7	83.1 74.1 73.6 69.7 74.1	C11	70.7 74.0 70.0 74.3 73.0
C2	81.0 81.9 82.8 76.3 83.4 86.1	C9	65.4 77.4 70.4 77.0 75.4 72.7
C4	86.9 81.2 84.3 84.5 82.5 76.9 80.4	C13	77.2 64.0 73.5 81.5 81.0 67.0 48.7
C6	83.0 80.2 84.6 86.7 83.5 86.4 83.4 83.8	C12	70.0 71.1 80.8 81.9 69.9 84.3 78.7 72.4
Initial count average, 81.4 Group average, 80.0		Initial count average, 74.6 Group average, 76.9	

cyte count in the last week was low, with a corresponding elevation of the polymorphonuclear count. The average in group II is lower than in group I, corresponding with the lower total leucocyte count and with the higher polymorphonuclear count.

An attempt has been made to differentiate between large and small lymphocytes (table 7). The initial counts of large lymphocytes in group I varied between 27.3 and 70.2 (average, 53.9 per cent). For all counts the average was 45.4 per cent. In group II the initial counts varied between 18.7 and 33.8 (average, 29.3 per cent) and all counts were between 18.7 and 40.1 (average, 29.1 per cent). Plasma cells were classed with lymphocytes. It is obvious from examination of the table that the counts are decidedly lower and more uniform in the second group than in the first. The explanation is probably in the greater age of the animals rather than in the change of technique (different source of blood).

TABLE 7

The large-lymphocyte percentage

Group I

RAT	PER CENT							
C3	61.9	27.6						
C8	27.3	33.5	38.1					
C5	61.2	45.3	52.9	35.3				
C7	41.9	34.1	34.8	47.8	31.2			
C2	59.0	64.5	45.6	53.7	27.6	40.0		
C4	70.2	40.8	63.5	51.7	31.5	37.4	47.5	
C6	56.2	48.2	53.7	61.7	36.4	48.8	32.7	44.7
Initial count average, 53.9								
Group average, 45.4								

Group II

RAT	PER CENT							
C15	32.3	32.1						
C14	21.7	25.2	38.5					
C10	32.8	27.3	29.5	40.1				
C11	18.7	30.9	19.4	31.2	35.0			
C9	33.7	35.7	34.1	30.2	23.2	23.0		
C13	31.8	33.7	39.8	37.8	36.7	27.1	23.7	
C12	33.8	16.8	23.6	37.8	26.4	20.5	28.6	31.8
Initial count average, 29.3								
Group average, 29.1								

The initial counts of large mononuclear cells and transitionals ranged from 0.4 to 2.4 per cent (average, 1.0 per cent) and from 0.2 to 6.5 per cent (average, 3.1 per cent) in the first and second groups, respectively. All counts in the first group ranged from 0 to 4.8 per cent (average, 1.45 per cent) and in the second group from 0.2 to 6.5 per cent (average, 2.6 per cent).

The results of other workers are given in table 8. The figures of Kleineberger and Carl ('12) are similar to ours. Happ ('22) and Laurens and Sooy ('24) obtained approximately 12 per cent more polymorphonuclear cells. Rivas ('14), Nowrey ('21), and Margot ('16) obtained entirely different results. An explanation of these discrepancies has been suggested by Donaldson ('24).

Several extraordinary forms of cells were encountered during the study. The most numerous of these were Rieder cells. On several occasions cells were seen which corresponded in most details to transitional cells, but having doughnut-shaped nuclei with a small central aperture. Three peculiar cells were seen in which no nucleus was visible. These were about the size of red cells and their cytoplasm was filled with large, uniform, eosinophilic granules, identical in appearance with those of the ordinary eosinophilic leucocyte. We have also seen ten small, nucleated cells, about the size of a red cell, whose cytoplasm was dull gray and contained numerous, small, irregular, reddish-blue granules. In one of these a considerable amount of brown, granular pigment was visible in the cytoplasm. The nucleus was, in most instances, indented, irregularly stained, and showed grayish-blue bands.

TABLE 8

Differential counts of other workers

AUTHOR	SOURCE OF BLOOD	AGE OF RAT	POLYS	N	E	B	S.L.	L.L.
Rivas	Tail	Adult	58.4	57	1.1	0.2	34.6	7.0
Rivas	Tail	Adult	53.4	52	0.6	0.3	41.7	4.9
Rivas	Tail	Adult	57.0	56	0.9	0.2	38.2	4.8
Nowrey	Tail	163 days	64.0	62	1.5	0.4	29.5	2.4
Margot	Tail	148 days	59.8	59	0.8		33.8	6.3
Kleineberger and Carl	Thigh	Adult	19.5	16	3.5		53.5	24.5
Happ	Thigh	4 months	30.0	24	6.0		68.0	
Happ	Thigh	9 months	30.0	26	4.0		68.0	
Laurens and Sooy	Tail	6 months	35.0	30	5.0		62.0	
Taylor		Adult	50.0		1.0		30.0	14.0

In some of the cells there were several nuclear bodies of various sizes resembling Howell-Jolly bodies.

The polynuclear counts of Arneth and Cooke

This study was made upon the same films used in the differential counts. Films stained by Wright's method were found entirely satisfactory when a reliable staining solution was used and even the finest nuclear filaments were readily seen. Heidenhain's iron-alum method as suggested by Cooke and Ponder ('27) gave no better results in our hands than did Wright's. Two methods of classifying the cells were used in each count. In the first we followed esssentially the method described by Arneth ('04), but the main classes were not subdivided. The cells were classified in five groups according to the number of distinct nuclear lobulations. This count was carried out simultaneously with the differential count and one hundred polymorphonuclear cells were counted in each instance. It was not until this study had been completed that we decided to reexamine all of the films and use the method of Cooke ('14) for classification. The criterion of that author was faithfully adhered to, namely: "if there is any band of nuclear material except this chromatin filament connecting the different parts of the nucleus, that nucleus cannot, for the purpose of the count, be said to be divided." Only fifty cells were counted in this study, since Ponder and Flinn ('26) have shown that the percentage of error is very small.

As one would expect, there is a great difference of distribution of the cells by the two methods. Certain difficulties in the classification arose which may, in part at least, explain why Kennedy ('27) found the rat unsuitable for this type of study. A large proportion of the nuclei are in the form of a closed ring. In many, including nearly all of the eosinophils, this ring is of uniform thickness; in others there is a varying degree of separation into lobules, connected by bands of different width. Cells with a uniform, ring-shaped nucleus were placed in class II in the Arneth tabulation.

In making the counts by Cooke's method the following criteria were decided upon. If there was no filament connection, the cell was placed in class I. If a single filament was present, it was placed in class II, etc. Examination of the accompanying illustrations (fig. 1) will show that in some cases, even when the filaments are present, the number of lobes does not correspond with the class. However, it seemed necessary to follow some such arbitrary system in order to obtain some degree of uniformity of results, since so large a proportion of the polymorphonuclear cells have ring-shaped nuclei.

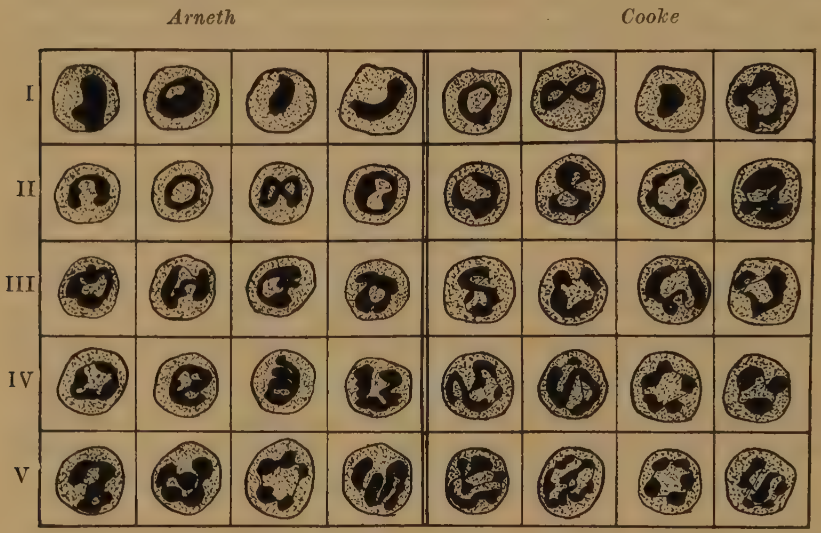

Figure 1

Eosinophil cells are included in all of the counts given. In the first thirty-five counts the values were computed both by including them and by omitting them. The values of the weighted means differed so little that for the present, at least, we decided to include them (table 9). Since the nucleus of practically every eosinophil consisted of an even ring, in our counts they fell into class II by Arneth's method and in class I by Cooke's. In addition to classifying the cells, the 'index' as suggested by Bushnell and Treuholtz ('08) and the 'weighted mean' of Cooke and Ponder ('27) were calculated. The results of the 'polynuclear counts' are given in table 9.

By Arneth's method the average values in the first group were 1, 27, 21, 28, 23 and in the second group, 1, 23, 25, 26, 25. The Bushnell and Treuholtz indices were 38 and 36 and the weighted means of Cooke and Ponder, 3.45 and 3.31, respectively. It will be seen that nearly all of the cells fall in the last four classes and are quite equally divided between them. The index showed great variation, with high and low limits in both groups of 66 and 15, respectively. The weighted mean varied between 2.38 and 4.64, a difference of 2.26, with 50 per cent of the counts falling between 3.62 and 3.68 (both groups) and 80 per cent between 3.15 and 3.88. In the first group when neutrophils only were included, the weighted means varied from 2.89 to 4.69—average, 3.50—and 50 per cent of the counts were between 3.52 and 3.76.

By Cooke's method the average count in group I was 73, 19, 6, 1, 1, and in group II, 68, 20, 8, 3, 1. The averages of the indices were 95 and 92 and the averages of the weighted means, 1.34 and 1.49, respectively. The majority of the cells, then, fell in class I, with a rapidly decreasing number in succeeding classes. The variation in the index was not as great as in the classification by Arneth's method; the limits in both groups were 84 and 99, with 50 per cent of the indices between 93 and 96. The weighted means varied from 1.13 to 1.76, with 82 per cent falling between 1.22 and 1.56. The weighted means of neutrophils alone varied from 1.21 to 1.61, with 50 per cent between 1.40 and 1.50.

We have found in the literature no reference to the Arneth count in rats. Cooke and Ponder have quoted Kennedy as stating that too great variations occurred for practical use. Cramer, Drew, and Mottram ('22) have noted that the polymorphonuclear cells in rats subjected to vitamine-A deficiency are less lobulated than normal, suggesting a change in the Arneth count.

The results of our observations show considerable uniformity in the actual counts and in the weighted means. Both

TABLE 9

The polynuclear count (group I)

Group I									*Group II*									
ARNETH									COOKE									
Rat	I	II	III	IV	V	Index	Mean[1]1	Mean[2]2	Rat	I	II	III	IV	V	Index	Mean[1]1	Mean[2]2
C3	0	24	20	38	18	34	3.50	3.53	C3	70	24	4	2	0	96	1.38	1.40
	0	24	10	40	26	29	3.68	3.70		68	20	12	0	0	94	1.44	1.45
C8	0	34	18	26	20	43	3.26	3.34	C8	80	18	2	0	0	99	1.22	1.23
	2	18	24	26	30	32	3.64	3.67		70	22	8	0	0	96	1.36	1.40
	4	16	16	28	36	28	3.76	3.79		80	16	2	0	2	97	1.28	1.30
C5	0	20	26	28	26	33	3.60	3.63	C5	76	18	4	2	0	96	1.32	1.34
	2	26	24	30	18	40	3.36	3.42		76	20	4	0	0	98	1.28	1.32
	0	26	24	26	24	38	3.48	3.54		70	24	6	0	0	97	1.36	1.41
	2	30	30	24	14	47	3.18	3.19		64	32	4	0	0	98	1.40	1.41
C7	0	32	14	42	12	39	3.34	3.52	C7	84	10	6	0	0	97	1.22	1.29
	0	56	14	20	10	63	2.84	2.97		80	12	4	4	0	94	1.32	1.44
	0	38	18	26	18	47	3.24	3.34		84	10	4	2	0	96	1.24	1.28
	2	48	24	14	12	62	2.38	2.97		84	8	8	0	0	96	1.24	1.33
	0	46	24	16	14	58	2.84	3.11		78	12	10	0	0	95	1.32	1.42
C2	0	20	16	36	28	28	3.72	3.75	C2	60	26	14	0	0	93	1.54	1.56
	0	12	24	32	48	24	4.64	4.69		72	16	10	0	2	93	1.44	1.46
	0	24	12	36	28	30	3.68	3.73		70	16	10	4	0	91	1.48	1.51
	—	—	—	—	—	—	—	—		—	—	—	—	—	—	—	—
	0	40	16	22	22	48	3.26	3.32		78	20	2	0	0	99	1.24	1.27
	2	56	16	12	14	66	2.80	2.89		86	12	2	0	0	99	1.13	1.21
C4	0	22	18	22	38	31	3.76	3.85	C4	72	18	4	6	0	92	1.44	1.49
	0	10	16	32	42	18	4.06	4.08		62	30	8	0	0	96	1.46	1.47
	0	18	18	34	30	27	3.76	3.83		68	22	2	8	0	91	1.54	1.59
	0	28	20	24	28	38	3.52	3.56		74	14	10	0	2	93	1.42	1.45
	0	52	20	18	10	62	2.86	2.96		82	14	0	2	2	96	1.28	1.36
	0	38	32	22	8	54	3.00	3.05		76	20	4	0	0	98	1.28	1.31
	0	28	14	36	22	35	3.52	3.58		60	26	12	2	0	92	1.56	1.61
C6	0	12	36	36	16	28	3.56	3.59	C6	70	22	4	2	2	94	1.44	1.46
	0	10	30	36	24	25	3.74	3.76		66	24	10	0	0	95	1.44	1.45
	0	12	18	38	32	21	3.90	3.92		68	18	10	2	2	91	1.54	1.53
	0	16	32	32	20	32	3.56	3.61		66	26	6	2	0	95	1.14	1.47
	0	20	24	36	20	32	3.56	3.57		74	24	2	0	0	99	1.28	1.29
	0	26	20	32	22	36	3.50	3.57		68	20	10	2	0	93	1.46	1.51
	0	22	20	36	22	32	3.58	3.63		72	20	8	0	0	96	1.36	1.38
	0	32	20	18	30	42	3.46	3.55		76	20	2	2	0	97	1.30	1.34
Average	1	27	21	28	23	38	3.44	3.45	Average	73	19	6	1	1	95	1.34	1.36

The polynuclear count (group II)

Group I *Group II*

	ARNETH								COOKE						
Rat	I	II	III	IV	V	Index	Mean[1]1	Rat	I	II	III	IV	V	Index	Mean[1]1
C15	1	39	20	20	20	50	3.19	C15	72	14	10	4	0	91	1.46
	0	37	15	24	24	44	3.35		68	20	6	4	2	91	1.52
C14	1	32	25	29	13	45	3.21	C14	54	34	12	0	0	94	1.58
	0	17	26	23	34	30	3.74		56	24	10	8	2	85	1.76
	2	9	24	22	43	23	3.95		62	32	4	2	2	96	1.46
C10	2	19	21	25	38	31	3.93	C10	58	30	10	2	0	93	1.56
	0	26	30	28	16	41	3.34		68	20	6	4	2	91	1.52
	0	37	23	18	22	48	3.25		80	14	6	0	0	97	1.26
	0	28	21	24	27	38	3.50		60	28	8	4	0	92	1.54
C11	3	32	23	29	13	46	3.17	C11	80	18	0	2	0	98	1.24
	0	10	27	35	28	23	3.81		60	20	18	2	0	89	1.62
	1	20	34	21	24	38	3.47		56	26	16	2	0	90	1.64
	1	18	27	24	30	32	3.64		80	14	6	0	0	97	1.26
	1	9	30	22	38	25	3.87		68	26	6	0	0	97	1.38
C9	—	—	—	—	—	35	—	C9	68	20	10	2	0	93	1.46
	1	15	25	33	26	28	3.68		80	12	2	6	0	93	1.34
	0	20	28	26	26	34	3.58		76	18	2	4	0	95	1.34
	2	24	22	27	25	37	3.49		72	22	3	2	0	96	1.36
	1	15	34	25	25	33	3.58		68	22	10	0	0	95	1.42
	3	25	30	27	15	43	3.26		82	10	6	0	2	95	1.42
C13	0	41	20	21	18	51	3.16	C13	72	20	6	2	0	95	1.34
	1	34	20	22	23	45	3.32		52	34	14	0	0	93	1.62
	1	9	11	16	68	15	4.56		40	16	14	16	14	63	2.44
	5	25	32	23	15	43	3.18		64	30	6	6	2	89	1.76
	1	35	18	23	23	45	3.32		74	10	8	4	4	88	1.54
	1	31	17	24	25	42	3.35		68	26	4	2	0	96	1.40
	2	10	31	22	35	27	3.78		62	24	12	2	0	92	1.54
C12	0	13	22	36	29	24	3.81	C12	68	22	6	4	0	93	1.46
	1	34	30	21	19	50	3.38		74	18	8	0	0	96	1.34
	2	45	24	21	8	59	2.88		82	8	4	6	0	92	1.34
	1	21	32	31	15	38	3.38		66	16	16	2	0	90	1.54
	0	23	25	30	22	35	3.51		64	26	8	2	0	94	1.48
	0	10	23	38	29	21	3.86		66	20	10	4	0	91	1.52
	1	14	25	25	35	27	3.79		70	26	2	2	0	97	1.36
	1	20	25	32	22	33	3.54		62	14	16	8	0	84	1.70
Average	1	23	25	26	25	36	3.31	Average	68	20	8	3	1	92	1.49

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[1] Weighted mean 1 is that computed from the total number of polymorphonuclear cells.

[2] Weighted mean 2 is that computed from the number of neutrophil cells.

Arneth's and Cooke's methods give sufficiently uniform results to be useful in experimental work, although the actual figures obtained by the two methods are quite different. The latter method seems to be more satisfactory, inasmuch as the criteria are more definite. On the other hand, it may be difficult to detect a 'shift to the left,' since there is even in the normal so large a proportion of cells in class I. Certainly, in expressing the results of the count by one number, Cooke's weighted mean is to be preferred. It can be seen in the table that when the results by Arneth's method were expressed by the index, there was great divergence between counts. This was much improved, however, when calculated from figures obtained by Cooke's method.

From the results given it would seem that there is a slight advantage in classifying only the neutrophilic cells, both in uniformity of results and in bringing the general picture somewhat to the right.

SUMMARY

Seventy leucocyte counts, absolute and differential, and Arneth counts have been made on two age groups of male albino rats (fourteen animals). The blood samples have been taken from different sources in the two groups, namely, from the femoral vein and from the tail. The results have been tabulated, compared, and discussed. The results of other workers have been tabulated for comparison with our own. We have described a method for applying Cooke's criterion for the classification of polymorphonuclear cells with ring-shaped nuclei.

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HYDROCEPHALUS IN LOWER ANIMALS

CONGENITAL OCCURRENCE IN A CALF AND AN ALBINO RAT

JOSEPH WILLIAM HOUCK
Laboratory of Histology and Embryology, Medical School, University of Virginia

SIX FIGURES

Although hydrocephalus is a familiar condition occurring rather frequently in infants and occasionally in adult man, one does not usually hear of it or think of it as occurring in other mammals. The anatomical structure of the lower mammalian brain is such that interference with the absorption of the ventricular fluid is theoretically just as possible and as probable as in the brain of man. Developmental errors and chance infections inducing the formation of hydrocephalus in domesticated animals, however, must be of such low incidence that usually the malformation proceeds unrecognized. The novel occurrence and recognition of the disease in two contrasting species within a short period of time seemed to warrant the study of the pathologic heads of the creatures so affected.

Experimental hydrocephalus has been produced in a variety of animals. Flexner ('07) recorded an occasional case of internal hydrocephalus in monkeys following the subarachnoid injection of meningococci. Dandy and Blackfan ('13, '14) produced typical internal hydrocephalus in dogs by introducing pledgets of cotton into the aqueduct of Sylvius. In one instance a similar dilatation of the ventricles was obtained by ligation of the vein of Galen. Young dogs two to six months old were used. Due to the previous closure of the sutures, however, no enlargement of the head occurred.

Thomas ('14) produced internal hydrocephalus in dogs by the intraventricular injection of the protein aleuronat in starch. Inflammatory blockage to the outward passage of the ventricular fluid usually occurred at one of the narrow passageways in the ventricular circulatory system. The subsequent intraventricular injection of India ink demonstrated that obstruction occurred either at the foramen of Monro, in the aqueduct of Sylvius, or, most frequently, at the foramen of Magendie.

Cushing ('08) found that two distinct types of internal hydrocephalus occurred in children. In the obstructive type the condition resulted from blockage in the ventricular system and could be drained only by means of ventricular puncture. In the communicating type, presumably due to blockage in the meninges, free communication was present between the ventricular system and the subarachnoid space, and drainage could be accomplished by lumbar puncture. Dandy and Blackfan ('13, '14) injected phenolsulphonephthalein into the lateral ventricles and demonstrated the lack of communication in the obstructive type. In such cases there was a negligible amount of absorption of the dye from the cerebral ventricles, but an impaired absorption from the subarachnoid space. In the communicating type subarachnoid absorption was markedly retarded.

Following up this work, Weed ('20) demonstrated experimentally the possibility of two types of hydrocephalus internus by a sterile injection of lampblack in Ringer's solution. Young kittens two months old were used. Great enlargement of the heads occurred, characterized by the same gross changes typically found in affected infants. Adult cats were also treated, but no cranial enlargement occurred, although the symptoms of cerebral irritation and the gross changes in the brain at necropsy showed that the hydrocephalic process was as easily produced in the adult. One adult cat showed no signs of acute cerebral pressure, but when sacrificed two months later, the lateral ventricles exhibited enlargement. However, the findings in individual subjects were very variable, as might be expected.

Weed used two methods of injection, intraventricular and subarachnoid (into the cisterna cerebellomedullaris). He states,

> There is no essential difference between hydrocephalus produced by intraventricular injection of lampblack and that produced by subarachnoid. Record has been made of minor differences, such as the more or less extensive obliteration of the third ventricle and of the septum pellucidum in the direct intraventricular injections, as compared with the partial survival of these structures in the subarachnoid type. Most marked of all the differentiations, however, is the variation in the distribution of the carbon particles found at autopsy. In the kitten receiving ventricular injection a dense black layer of granules obscuring the picture is customarily found in the basal regions of the dilated cerebral ventricles. In the upper half of the cavity, however, the amount of lampblack is much less. Quite unlike this is the much smaller amount of carbon visible in the dilated ventricles injected by the subarachnoid route. In these, collections of granules, scattered and in small amount, are the usual findings in the ventricular floor, but in some specimens the walls of the ventricles are obscured by a deposit giving in the gross a brownish tint.

From this work and from previous work already cited, we may conclude that for any marked enlargement of the head to occur, the increased intracranial pressure must be present early in developmental life, either prenatally or very soon after birth. On the basis of the collective evidence from these various papers upon hydrocephalus internus, we may conclude further that hydrocephalus is readily produced in mammalian forms and that it may follow either ventricular block or a diffuse meningeal block. There is very little difference and possibly no constant variation in the changes produced in the skull and in the brain as a whole by the two types. Furthermore, enlargement of the skull, the degree of expansion and the nature of the disfiguration may be used as indices of the approximate age in pathogenesis, since such alterations depend directly upon the state of fixation of the sutures of the cranial bones and upon their stage of development and ossification. We may conceive of hydrocephalus in the fetus and in the newborn as resulting from one of two causes: developmental errors in the ventricular system or in the meningeal absorptive mechanism, and damage to the cerebrospinal system caused by an inflammatory process accompanying meningeal or ependymal infection in the ventricular canal.

HYDROCEPHALIC CALF

On April 15, 1927, a large calf was killed at the Charlottesville abattoir. When the butcher opened the head to remove the brains, he was astonished to find only 'a thin bag of water.' The head was secured by Dr. H. E. Jordan and preserved in 10 per cent formalin.

The calf had been sold for veal by a farmer living in this vicinity and, together with employes of the local abattoir, he supplied fragments of the history of the animal. The very large head was noticeable at birth and increased in size in proportion to increase in size of the body. The ratio of size of head to size of body remained approximately constant after birth, but the head was considerably larger than normal. The mentality and physical development of the animal were apparently normal and no strange symptoms were observed during its peaceful life on the farm. On the contrary, the breeder was proud of the calf and certified to its intelligence and tranquil nature. The butcher considered it a fine 'veal.' The carcass weighed 205 pounds when killed at the age of eight weeks, indicating a large, unusually well-developed body.

Investigation of the calf's antecedents revealed that the father was a Durham bull with a noticeably large head. This, together with the fact that the horns were said to be far apart, suggests that a low grade of hydrocephalus may have existed in the male parent. No other abnormalities could be traced in the family tree. The bull has been sold and its whereabouts is unknown. The mother of the calf was a half-breed Jersey-Hereford.

On examination, the skull and brain were found so distorted that perfect function would seem impossible. In this connection the butcher offered one fact which he considered a mark of intelligence: the calf would not walk across a narrow crack in the slaughterhouse floor, but balked obstinately and had to be half-lifted over the 'obstruction.' This suggests that the calf was functioning as a reflex animal, because of the damage to the higher centers, along with some disturbance of the optic apparatus. One cannot readily attribute normal function to such a disfigured brain, yet we have the statement of the breeder that the animal was unusually intelligent and also the fact of exceptional physical development which always depends on a reasonably well-functioning nervous system. Cushing ('11) observed, however, that hydrocephalic infants were usually more than well nourished. The weight of our subject (205 pounds) seemed excessive. From questioning several breeders it was estimated that an eight weeks' calf should weigh between 160 and 180 pounds.

THE SKULL

The head was examined after formalin fixation. The 'skull cap' portion of the cranial vault had been cut away by the butcher in his explorations, thereby allowing free contact with the fixing fluid. Externally, the large size of the head was the outstanding pathologic feature. The calvarium was expanded and rounded out in a dome-shaped caricature, with the horns and ears widely apart. The head bulged chiefly in the frontal and temporal regions. In spite of this enlargement, the bones of the head were quite firm and the muscles well developed.

The iris of the eye seemed directed downward, showing a white line of sclera above the iris, but none below. Later examinations of the scraped skull revealed that the shape of the orbit was markedly changed; the orbital roof elevated and the volume of the orbital cavity considerably increased. Weed ('20), working experimentally with kittens, described this peculiar appearance of the iris and attributed it to the pulling upward of the facial skin by the bony expansion of the skull. Thomas ('23) referred to the frequency of this phenomenon, but stated that the eyes are directed upward in human babies. It seems that an expansion of the head upward and forward would tend to direct the eyes downward.

When the 'skull cap' was removed, a view of the interior of the brain was obtained. As the cerebral cortex had been reduced to the thickness of blotting-paper by the hydrocephalic process, the roof of the brain came away with the 'skull cap.' The interior appeared as one large chamber, the shape and size of the cranial cavity, and gave the appearance of being delicately lined with a membrane of nervous tissue. At the base of this cavity, which represented the enlarged ventricles, the basal ganglia stood out in bold relief.

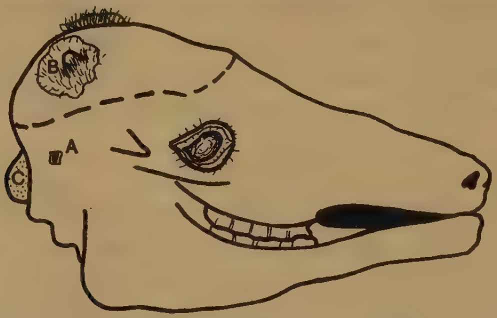


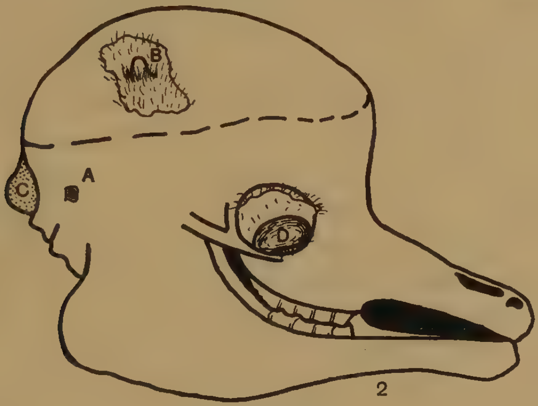
Fig. 1 Skull of normal calf (side view). A. External auditory meatus. B. Horn and patch of skin. C. Occipital condyle.

A drawing (fig. 4) of this view of the distorted brain in situ will be discussed below when the brain is described.

A normal calf's head of approximately the same age was obtained from the abattoir and compared with the hydrocephalic specimen. The essential differences in the two were confined to the bony skull, so both skulls were cleaned, scraped, and dried. The brain of the normal head was fixed in 10 per cent formalin for comparison.

Compared with the normal (fig. 1), the hydrocephalic skull (figs. 2 and 3) had lost the usual triangular shape and consisted of a dome-shaped cranial vault, with projecting snout, otherwise approaching somewhat the shape of the human skull. The greatest changes occurred in the bones composing the calvarium, but few of the cranial bones escaped some alteration.

The dome of the skull was spread out apparently to accommodate the increased intracranial tension. The spreading



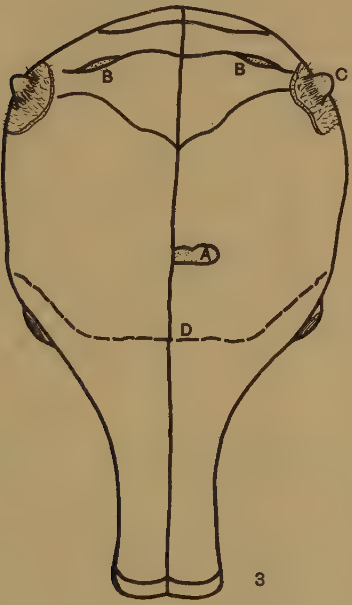


Fig. 2 Hydrocephalic skull of calf (side view). A. External auditory meatus. B. Horn and patch of skin. C. Occipital condyle. D. Partly obscured pupil.

Fig. 3 Hydrocephalic skull of calf (from above). A. Membranous plaque of thin bony structure. B. Membranous portion of parieto-occipital suture. C. Horn and patch of skin. D. Line of cut for removing skull cap.

involved an increase in the surface area of the frontal, parietal, and occipital bones, accompanied by considerable thinning. At one point on the frontal bone to the left of the sagittal suture, a paper-thin membrane was all that protected the brain within. The sutures were well formed along most of their course, except for a membranous fontanelle on each side in the parieto-occipital suture (fig. 3, *B*). In general, the bones of the skull were remarkably intact and solid, in view of the great dilatation of the cranial fossae. The greatest hyperplasia was in the frontal bones which formed the bulging forehead. The skull was not quite symmetrical. The bulging was slightly greater on the left side.

The interior of the skull gave the appearance of compression. It was smoothed out by pressure, and most of the structures which usually project into the cranial cavity were flattened to the point of obliteration. The orbital bones were pushed out of the interior, their walls thin, even membranous in spots. The sphenoid bones and the base of the skull were flattened out. The frontal sinuses which are very prominent in the normal skull were completely obliterated. The furrows in the interior of the skull which ordinarily correspond to convolutions of the brain and the blood vessels of the meninges were ironed out by the pressure. The whole interior of the skull was widened; the fossae were increased, and the total volume of the cranial cavity was augmented to four or five times the normal. The greatest changes appeared to have occurred in the bones of membranous embryological development.

For comparison, measurements of both skulls were taken. The table of measurements indicates the extent of expansion in the hydrocephalic specimen, and also the sites where the expansion occurred. The mandible, hyoid bones, and occipital condyles are of practically the same size in both normal and pathologic specimens. The other measurements, however, illustrate the gross differences.

Skull measurements (in centimeters)

	NORMAL	HYDROCEPHALIC
1. Mandible, angle to tip	21.4	23.2
2. Hyoid, greater cornu to thyroid cornu	7.8	8.0
3. Occipital condyles, greatest width lateral margins	7.3	7.2
4. Orbits, shortest distance between	9.9	14.7
5. Calvarium, greatest width (above orbits)	12.0	16.4
6. Horns, distance between	8.4	13.7
7. Skull, greatest length (tip of nose to occipital protuberance)	25.2	31.7
8. Circumference (skull cap)	43.1	58.1
9. Gonion to bregma	19.0	26.9
10. Nasion to bregma	11.2	16.0
11. Orbit, diameter	4.5	5.0
12. Greater wings of sphenoid, distance between tips	7.5	12.0

THE BRAIN

Figure 4 represents a view of the hydrocephalic brain in situ, with the top of what constituted a single large cerebral vesicle removed. Because of a high intracranial pressure, presumably over several months' time, the lateral ventricles had been tremendously dilated; and with the loss of the septum pellucidum and the greater part of the corpus callosum, the several cavities had coalesced into a single large ventricle. All other changes center around this dilatation of the lateral ventricles.

The structures which normally lie in the floor of the ventricular cavities were found intact, but somewhat flattened out over the base of the skull; they stood out sharply, due to compression of the surrounding cerebral tissue. The corpora striata, mammillary bodies, and hippocampi could be identified in the floor of the vesicle. The choroid 'glands' of the lateral ventricles were plainly discernible. In the superior portion of the floor of the vesicle were two extensions of the main cavity into the rhinencephalon, forming an enlarged rhinocele. The lateral walls of the cerebrum, including cortex and medulla, were reduced to the thickness of a few millimeters. Bisecting the center of the vesicle was the incomplete remnant of the medial portions of the cerebral hemispheres.

By turning aside a flap of the posterior floor of the cerebral vesicle and likewise the tentorium cerebelli, it was possible to expose the cerebellum, the corpora quadrigemina, and other structures of the base of the brain. They were apparently in good order. The brain and meninges were then removed from the skull. The dura was firmly adherent to the sutures of the skull. The hypophysis cerebri was removed for special study.

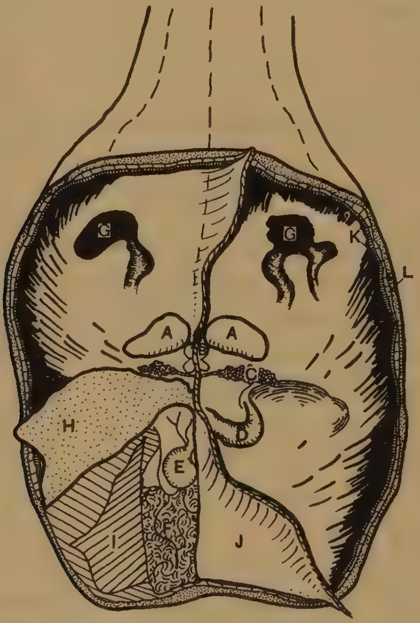


Fig. 4 Hydrocephalic brain of calf in situ (skull cap removed). A. Corpora striata. B. Anterior pillar of fornix. C. Choroid plexus of lateral ventricle. D. Hippocampus. E. Corpora quadrigemina. F. Cerebellum. G. Rhinencephalon (rhinocoele). H. Flap of cerebral hemisphere turned aside. I. Flap of tentorium cerebelli turned aside. J. Median septum. K. Wall of ventricle (cerebral hemisphere) cross-cut. I. Dura mater.

The ventral aspect of the brain (fig. 5) showed some of the structures of the brain stem, which seemed undamaged, except for some lengthening and flattening, paralleling the changes in the skull. The rhinencephalon and optic tracts were apparently in continuity with the rest of the brain. The mammillary body, which is single in the bovine species, the pons and medulla, and the roots of the cranial nerves appeared to be intact. The cerebellum seemed slightly enlarged.

For a more detailed study of the brain stem, a sagittal section was made (fig. 6). Most of the structures of the brain stem could be identified. The pons, however, was noticeably flattened together with the rest of the brain stem. The lamina quadrigemina and the fourth ventricle appeared fairly normal, but the structures rostral to the lamina quadrigemina had a peculiar configuration. The pineal body was

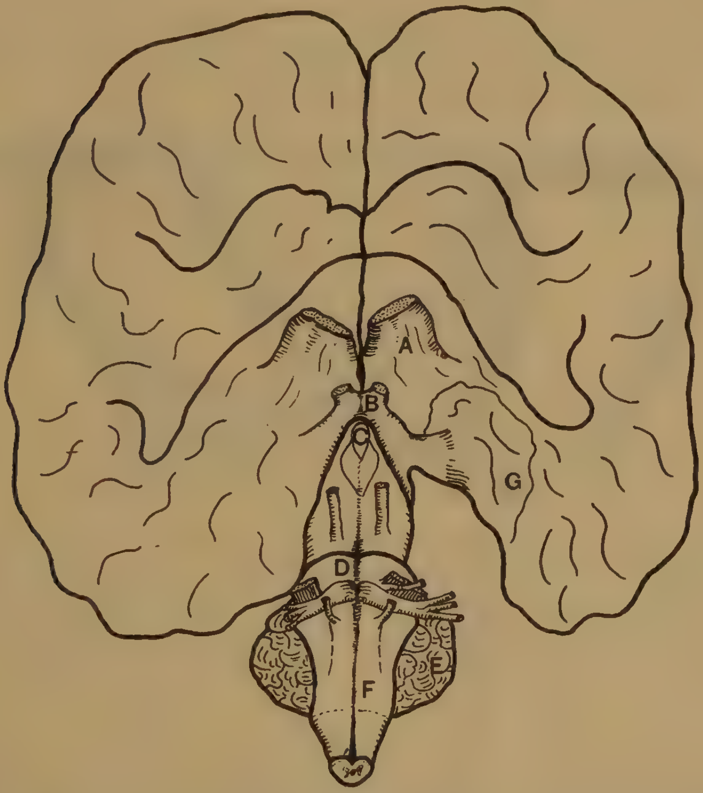


Fig. 5 Ventral view of hydrocephalic brain of calf. A. Rhinencephalon (olfactory bulb). B. Optic chiasm. C. Mammillary body. D. Pons. E. Cerebellum. F. Medulla oblongata. G. Flap of cerebral tissue turned aside.

absent and only its stalk remained. The intermediary substance between the thalami (massa intermedia) constituted a very large area in the section. Almost the whole portion of the thalami seemed to be fused. The third ventricle was obliterated, except for a very narrow channel. A probe could be passed through the interventricular foramina, which were extremely narrow slits subjacent to the flattened anterior pillar of the fornix. Part of the body of the fornix remained and was continuous with the fimbria of the hippocampus and the alveus. The anterior commissure was plainly visible. The cerebellum was pushed down firmly against the medulla oblongata and close to the corpora quadrigemina. Part of the splenium of the corpus callosum was apparently in place and also the posterior commissure. The hippocampus was flattened down on the floor of the lateral ventricles. Fiber bands which stood out from the rest of the cerebral medulla in distinct strips represented radiations of fibers from the corpora striata and thalami, to the cerebral cortex (figs. 4 and 6). The choroid glands of the lateral ventricles were very promi-

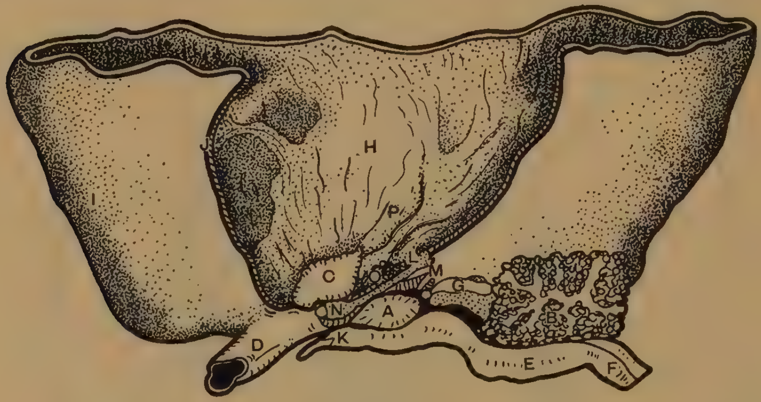


Fig. 6 Sagittal section of hydrocephalic brain of calf. A. Massa intermedia. B. Cerebellum. C. Corpus striatum. D. Olfactory bulb. E. Pons varolii. F. Medulla oblongata. G. Lamina quadrigemina. H. Cerebral medulla. I. Cerebral cortex (medial wall of hemisphere). J. Junction of cerebral hemispheres (corpus callosum). K. Optic chiasm. L. Body of fornix. M. Stalk of pineal body. N. Anterior pillar of fornix and anterior commissure. O. Choroid plexus of lateral ventricle. P. Fiber bundle.

nent. The corpus callosum was probably pushed to the roof of the vesicle.

The third ventricle appeared to have been obstructed by growth between the thalami. Although apparently not completely occluded, very imperfect drainage could have existed. The peculiar appearance of the elements of the diencephalon suggested that some of the missing structures had never formed. The hydrocephalic process presumably started early in fetal life, and development proceeded along a new plan because of the increased pressure. The cerebral tissue was spread out, leaving intact only the more resistant portions of the brain, which incidentally were those most necessary for the function of the organism. The cerebral gyri and sulci were completely obliterated.

Microscopic sections of the diencephalon and of the walls of the lateral ventricle were prepared and stained with Delafield's hematoxylin and eosin. Since the walls of the lateral ventricle were thinned to several millimeters, the sections include both cerebral cortex and medulla in a small area. In these sections the nerve elements of the ventricular walls appeared greatly compressed and the cells and fibers were arranged in strata and closely packed. There was only a minimal amount of destruction. Some loss of nerve cells had occurred along the whole extent of the roof of the lateral ventricles, probably the result of pressure atrophy, but the destruction was progressively less toward the region of the brain stem where the cells were approximately normal in appearance. The damaged cells in the upper cortex were either shrunken or else small cavities remained, due to lysis of the neuroplasm of the individual scattered cells. Weed ('20) remarked that, in kittens with a thinning of the cerebral cortex in marked hydrocephalus, there is a rearrangement of the nerve elements with a negligible destruction of cells and fibers. He based his conclusion upon macroscopic examination. Our microscopic sections confirm this view. The functional continuity of the rearranged cells and fibers explains how the animal could carry on in a normal manner with what was apparently a greatly damaged brain. Portions of the upper cortex were edematous.

A section through the diencephalon at the level of the foramen of Monro indicated patency of this foramen, strands of the choroid plexus being visible along the extent of the slit-like aperture. The ependymal lining of the greatly narrowed third ventricle was almost completely eroded, but small alveolar pockets of ependymal growth surrounded the lumen, having apparently burrowed by overgrowth into the neighboring neural tissue. These outgrowths were interpreted as a response, perhaps largely passive, to the pressure exerted upon the greatly decreased outlet. The choroid plexuses of the lateral ventricles were markedly distended. The choroid vessels were congested, presumably from back pressure. Thomas ('14) recorded an ependymal overgrowth in several of his cases of experimental hydrocephalus, in one case in the cerebral aqueduct, in a region narrowed like the one in our specimen.

PATHOGENESIS

From the anatomical description of the diencephalon, one might assume that the retention of ventricular fluid resulted from developmental malgrowth of the tissues which composed the walls of the third ventricle, thereby producing a block to cerebrospinal circulation at this point. There was a very great enlargement of the massa intermedia, and the lumen of the third ventricle was narrowed to the dimension of a millimeter in its most constricted portions. In spite of its extreme contraction, the third ventricle afforded potentially a continuous channel for the flow of cerebrospinal fluid.

The condition of the foramina of Luschka and Magendie could not be determined. It is possible that the pathologic process was initiated through lack of their patency and that the other changes were wholly secondary. The flattening down of the anterior pillar of the fornix and the related structures to occlude the foramina of Monro and the closure of the third ventricle might well be changes which followed the ensuing increase in intraventricular pressure. The evidence at hand is not decisive. But the entire base of the brain is unmistakably compressed.

It is possible that the communicating type of hydrocephalus was present at first. From the work of Weed ('20), it appears that a diffuse injury to the subarachnoid absorptive mechanism may produce the same pathologic picture as in the specimen at hand. He says:

> The gross pathologic lesion in kittens surviving the intradural injection of lampblack for ten days or over is practically identical in the different specimens. Reduced to simplest form, the abnormality consists in a tremendous and remarkable dilation of the cerebral lateral ventricles, associated with an enlargement of the kitten's head, and results in a marked thinning of the cerebral cortex. In some of the cases the third ventricle seems obliterated by its marked enlargement and by the rearrangements of the walls of the interventricular foramina: in others the form of this ventricle is still left, though the whole structure has greatly increased in all of its dimensions. The underlying basal nuclei seem to survive this experimental increase in cerebro-spinal pressure most efficiently; their markings are still plainly visible in the basal view of the sectioned specimen. These general characteristics hold for practically all the specimens obtained. The whole process may be likened to a partial reversion to the embryonic ventricle.

The several possibilities necessitate the classification of this case as one of so-called 'essential hydrocephalus,' gradually developing during fetal life and of uncertain causation. The malformation of the walls of the third ventricle were so great, however, that one must incline toward considering this region as the starting-point of the trouble.

An approximate estimate of the embryological stage at which the process started can be made. Weed ('17) related the first intraventricular flow of cerebrospinal fluid to the initial tufting of the choroid plexus. In the pig embryo the first extension of ventricular fluid into the periaxial tissues occurs at the 14-mm. stage. When the 26-mm. stage (end of second month) is reached, the periaxial spaces are completely filled. A congenital hydrocephalic process would therefore be initiated between the end of the second month and parturition. However, to estimate accurately the age at which malformation started, one would have to be entirely familiar with the etiology of the case. Presuming that the process started at such an early age, one cannot but doubt that some of the missing structures of the hydrocephalic brain ever existed in normal form. This applies to the corpus callosum, septum pellucidum, and the malformation of the region of the third ventricle. The analogy of reversion to embryonic form is a fairly close one in this specimen.

'Essential hydrocephalus' might develop as the result of a belated formation of the subarachnoid spaces. Lack of these spaces during the development of the choroid plexuses would furnish no provision for the absorption of the earlier secreted fluid. The secondary changes due to this initial imbalance might perpetuate the faulty absorption by a flattening of the embryonic brain stem. In any case, the changes follow a lack of balance between secretion and absorption. When absorption is decreased (never entirely prevented), a new balance is formed at a higher pressure. The gradient of pressure from within the ventricles outward must be downward as in any fluid system, thereby explaining how internal hydrocephalus may follow an intrameningeal 'block,' when an external hydrocephalus might be expected. The intracranial tension must certainly be a factor in determining the size of the head and brain of the individual embryo.

The changes in a brain following dilatation of the ventricles are too variable to warrant any more detailed discussion. As in the calf's brain, the whole brain is rearranged by a spreading out of its tissue in a thin structure, which can often be accompanied by reasonably normal function.

THE HYPOPHYSIS CEREBRI

The hypophysis was removed from a considerably flattened sella turcica. The gland was surrounded by a hemorrhagic sinus, which may have been the result of enlargement or rupture of the circular sinus into the capsule of the pituitary body. When compared with a normal specimen, the hypophysis was noticeably enlarged and flattened from the usual globoid shape into a broadened thick disk. Celloidin sections were prepared, together with sections of a normal hypophysis. The sections were stained with Delafield's hematoxylin and eosin.

Examination of the sections macroscopically revealed that the posterior lobe in the hydrocephalic specimen was greatly decreased in size—to approximately one-sixth to one-eighth the normal. The size of the anterior lobe, as estimated from its cross-section area, did not appear greater than the normal, rather slightly less. The gross examination was deceptive, because of the disk-like flattening. The true change in the gland was a flattening with some decrease in total volume. This change, together with the flattening of the sella turcica, was, of course, the result of high intracranial tension.

Microscopically, very little change was discerned in the structure of the pars neuralis. Its substance seemed dense and stratified and its capillary blood supply less regular. The residual lumen was obliterated. The pars infundibularis of the intermediate portion exhibited marked change. The dense stratum of faintly basophilic cells was increased in thickness and the arrangement characterized by irregularity. This mass merges into the substance of the pars distalis, normally separated by the residual lumen.

Eosinophilic cells predominated in the parenchyma of the pars distalis as they do in normal sections. However, both types of cells appeared to take a deeper stain than usual. The entire anterior lobe was stratified by pressure, and some of the layers were composed almost entirely of one type of cell, either acidophil or basophil. In addition to this change, small nodular growths of eosinophils with a sparse blood supply were seen studding the mass of compressed cells. The acinous structure characteristic of the pars distalis was lacking and the cells were aggregated compactly. A great number of the cells were multinucleated, a pathologic change which, according to Bailey and Davidoff ('25), is constantly found associated with hypophyseal adenoma and acromegaly. Ewing ('28) cites Erdheim as having observed adenomas of chromophil cells in four cases of gestation hypertrophy.

As stated above, our hydrocephalic specimen was well-nourished and from all indications distinctly overweight. In attempting to correlate this fact with the changes in the hypophysis cerebri, the following conclusions are suggested:

1. The animal in question showed evidence of acromegaly. It was well overweight, and the skull showed great development with a large thick jaw-bone and heavy basal skull bones (fig. 2).
2. The associated change in the hypophysis was similar to that described as the typical finding in acromegaly (Bailey and Davidoff). The change appeared to have been primarily a functional hyperplasia with some adenomatous overgrowth of eosinophil cells. Pressure was presumably the exciting factor.
3. The changes which occurred in the pars intermedia and in the location of the obliterated residual lumen cannot be interpreted, but possibly may represent the beginning of a basophil cell overgrowth.
4. The changes in the gland were undoubtedly functional. There was no increase in size and no evidence of neoplasia, except the borderline hyperplasia.

THE PINEAL BODY

The epiphysis cerebri was destroyed along with the corpus callosum and septum pellucidum by the hydrocephalic process. Since the physiologic importance of this body is in great doubt, the effect of this accidental pinealectomy cannot be estimated. The fact that the calf was apparently in excellent health and a fine physical specimen seems to suggest that the pineal body is not of vital importance to the organism.

Jordan ('11, '12) is of the opinion that if the pineal of sheep subserves any important function at all, it is for only the first eight months of postnatal life. Our specimen came within this range. Various series of pineal extirpations have been performed upon mammals (e.g., Izawa, '23). The collective evidence indicates that it plays some rôle in the development of puberty and possibly in the early growth of the body. Concerning the functional interrelationship of the pineal and hypophysis, the work of Jordan and Eyster ('11) suggests that the pineal secretion may be antagonistic to or inhibit postpituitary activity.

Our case shows association of the absence of the pineal with atrophy or decrease in the posterior lobe of the hypophysis and with what appears to be hyperplastic overgrowth and increased function of the anterior lobe. It is unfortunate that the external genitalia of the animal could not have been studied. No physiologic correlation could be determined.

The following conclusions are suggested:

1. The pineal is not essential to life and health.
2. If the body subserves a function during early postnatal life, even at this time its functions are probably unessential for the well-being of the organism.
3. Functional interrelation of the pineal with the other ductless glands is possible, but no definite relation can be traced in this case.

RELATED HUMAN CASES (Cushing, '11)

Cushing describes a case (XXXVIII) in which acromegaly was associated with internal hydrocephalus and a cerebellar cyst. The hydrocephalus was the result of pressure from the cerebellar cyst. The hypophysis was enlarged with the anterior lobe soft and necrotic. The anterior lobe was completely degenerated, but took a heavy diffuse eosin stain. The pars intermedia showed a marked hyperplasia (as in the case of the hydrocephalic calf) with invasion of the posterior lobe.

Case XXXIX (Cushing) shows association of an internal hydrocephalus of some years' duration with acromegaly. The hydrocephalus was of slow progress and relieved by decompression. Cushing states that flattening of the pituitary body and dyspituitarism frequently accompany hydrocephalus. When symptoms of pituitary disorder can be discerned in the hydrocephalic syndrome, hypopituitarism is the more common. The two cases of acromegaly with hydrocephalus referred to above are the only ones cited of hyperpituitarism. He considers the hydrocephalus the primary cause of pituitary dysfunction in these cases. Numerous cases of pituitary and pineal involvement with hydrocephalus are cited. Most of the pineal lesions are tumors.

HYDROCEPHALIC ALBINO RAT

This specimen was one of a litter of ten white rats born in Doctor Jordan's colony at the University of Virginia Medical School. The mother had been fed for two months on a diet of carbohydrate, which was continued up to and after the birth of the litter. The other nine of the litter, which was a good winter yield, were apparently normal in every particular.

The fifth day after birth, signs pointing to hydrocephalus were noticeable in one of the offspring. The head was noticeably large and the animal was considerably sluggish in comparison with its mates. Evidence of nervous instability was noticed; the movements and locomotion were uncertain, and there was a tendency to walk in circles. The symptoms became progressively worse until the twenty-fifth day after birth, when the animal died.

The specimen was received March 27, 1929, at the age of twenty-five days. A study of its reactions, posture, and habits was made immediately previous to its death. The following observations were made:

1. The rat lay in a hunched-up position with its belly and nose resting on the floor. The limbs appeared too weak to support the body and the neck too weak to support the head.
2. The lower lid was pulled up, completely obscuring the pupil (a universal sign found in newborn hydrocephalics). The animal did not react to movements in front of the eyes (noxious stimuli), indicating blindness.
3. The head was noticeably enlarged in the frontal and parietal regions, giving a dome shape to the head which would ordinarily be flat.

4. The weight was 27 grams, as compared with an average of 54 grams for the rest of the litter.

5. The feces were hard and adherent to the anus, and the belly was wet from the discharge of urine.

6. The undisturbed state was one of weakness, somnolence, and passivity.

7. When touched or pinched on any part of the body, the response was a circular movement to the right. The rat seemed very sensitive to cutaneous stimuli, quivering when touched, but the motor response was slowly effected and ataxic. The movement to the right was invariable, in spite of rotation in different planes (while in a pan).

8. The rat was carried 100 yards in a pan. During this journey it attempted to climb over the 2-inch edge of the pan. It managed to raise its hind quarters above the edge, but its head was too heavy to negotiate the escape.

9. The animal fell to the right occasionally, regaining its feet with difficulty. It was placed on its back several times, but could not be kept in this position; it quickly pawed its way to the prone posture.

10. The limbs were in a state of hypertonus, and the body quivered continually, more so when stimulated.

11. Respiration was rapid (112).

12. Several times it made agonal movements; it tucked the head between the fore legs and once almost stood on its head.

13. The body was undernourished. History showed that blindness had prevented the finding of food and water several days previous to death. In addition to this, physiologic wasting must have occurred from the hypertonic state of the muscles.

After death, the head was skinned, and the skull, which was soft and pliable, was opened with scissors. The interior of the cranial cavity was a mass of fluid, as had been previously observed in the calf. The cerebral tissue had been so flattened by intracranial pressure that it was paper-thin and closely adherent to the membranous cranium. The cranial vault, with the adherent cerebral tissue, was turned aside in four flaps, revealing internally an open cavity with the ganglia and other structures of the brain stem in sharp relief at the base of the distorted brain. The entire mass of tissue which survived the hydrocephalic process of destruction so resembled the brain of the calf, previously described, that it could almost be considered a miniature model of the same. The corpus callosum, corona radiata, septum pellucidum, most of the fornix—in fact, practically the entire structure (including the interconnections) of the cerebral hemispheres—were lacking. The animal was essentially a decorticate preparation. The only structures of the cerebrum remaining in good condition were the basal portions of the corpora striata.

The brain stem appeared to be almost completely intact. The pineal, hippocampi, corpora quadrigemina from the dorsal view afforded by the atrophic dissection appeared to be in correct apposition with the rest of the base of the brain. The cerebellum was displaced posteriorly and somewhat herniated into the foramen magnum. This may account for some of the medullary symptoms.

The abdominal and thoracic viscera were examined for possible anomalies, but the findings were negative, except for the thymus, which exhibited marked atrophy. The intestines were practically empty, accounting for the undernutrition which was to be expected from the history.

I wish to express my gratitude to Dr. H. E. Jordan for this material, for constant guidance in its study, and for helpful suggestions in the preparation of this paper. I am indebted also to Alice Clarke Mullen for assistance in the preparation of the illustrations.

SUMMARY

1. Two cases of spontaneous congenital hydrocephalus are described. The pathologic changes in both cases are very similar and parallel the changes produced experimentally in mammals by a number of workers. The findings are comparable to those found in human hydrocephalics.

2. The brain of the hydrocephalic calf was considerably deformed by increased intracranial tension. The abnormal morphology stands in paradoxical relation to the history of apparently normal behavior. Microscopic sections indicate a minimal destruction of nerve elements. A stretching and rearrangement of fibers and cells had occurred, but without obvious loss of functional continuity.

3. The location of the primary lesion in the brain of the calf could not be definitely established. However, the hydrocephalus had apparently resulted from a congenital malgrowth in the diencephalon with a constriction of the third ventricle to a very narrow canal.

4. The histologic changes which, according to Bailey and Davidoff, accompany acromegaly were seen in sections of the hypophysis cerebri of the calf. This diagnosis is further supported by the excess weight of the animal and the strong overdeveloped bones of the skull. The obliteration of the pineal body is offered as evidence of the functional unimportance of this structure to the organism, even in the early stages of development.

5. The head of the hydrocephalic rat is a miniature model of the calf's head. The history of the animal is given in detail and contrasts with that of the calf in that the rat showed symptoms of marked nervous derangement. It died of starvation, due to its inability to find food or water. The damage to the brain appeared, on gross examination, to be greater in the case of the rat, and its agonal death points to extensive destruction in the higher centers. The primary lesion producing the condition could not be determined, because of the small size of the brain and the delicacy of the compressed tissue.

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EDITORIAL

MOTION PICTURES AS AN ADJUNCT TO THE TEACHING OF MEDICAL SUBJECTS

(EXTRACTS FROM STATEMENT PREPARED BY COMMITTEE OF AMERICAN COLLEGE OF SURGEONS)

The value of motion pictures for medical instruction is gradually becoming more appreciated. For the student they can never replace experience gained from personal contact with disease, nor can they supplant the present well-established methods of teaching medicine. But as an adjunct to the methods in vogue at present, by facilitating the instruction, conserving the time of instructors and students, and by economy of materials, they are of inestimable value. Naturally, such statements presuppose high-grade production. Accuracy as to scientific detail, good cinematographic technique, and the best of photographic quality are primary requisites. With these qualities incorporated, motion pictures acquire considerable value in all branches of medical science.

Appreciating the value of motion pictures for medical instruction, whether for the student or postgraduate, Eastman Teaching Films, Inc., in collaboration with the American College of Surgeons, has undertaken productions along the lines described. Improved technique, improvement of subject content, and careful scientific supervision combine to make these films preeminent in the field of medical motion pictures. It is the determination of their proper application, which, to a large extent, governs the production of Eastman Medical Films.

THE ANATOMICAL RECORD, VOL. 45, NO. 2
MARCH, 1930

Up to the present time, the following films have been prepared:

1. The technique of blood transfusion (prepared at the University of Rochester School of Medicine).
2. Intestinal peristalsis (Drs. Walter C. Alvarez and Arnold Zimmerman, Mayo Clinic).
3. Indirect inguinal hernia (Dr. Daniel L. Borden, George Washington University Medical School).
4. Rabies (Dr. Julius Hess, Cook County Hospital, Chicago).
5. Diagnosis and treatment of infections of the hand (Dr. Allen B. Kanavel, Chicago).
6. Ectopic heart (Kansas City General Hospital).
7. Benign prostatic hypertrophy (Dr. J. Bentley Squier, Columbia).
8. Amyotonia congenita.
9. Simple goiter (Dr. George W. Crile, Cleveland).
10. The normal heart.
11. The treatment of a normal breech presentation (Dr. Joseph B. De Lee, Chicago).
12. The development of the fertilized rabbit's ovum (Dr. Warren H. Lewis, Carnegie Institute of Embryology).
13. Tests of vestibular function (Dr. Richard Lyman, University of Rochester).
14. Acute appendicitis (Dr. Edward Martin, Philadelphia).

Information pertaining to the rental or purchase of these films may be obtained from the Eastman Teaching Films, Inc., Rochester, New York.

THE ORIGIN OF THE FAT-BODIES IN AMBLYSTOMA MACULATUM

GLADYS K. McCOSH
Zoölogical Laboratory, Cornell University, Ithaca, New York

TWO PLATES (NINE FIGURES)

INTRODUCTION

Two views have been advanced regarding the origin of the fat-bodies in Amphibia: one, that the corpus adiposum and the gonad are derived from a common ridge, the other, that the fat-body arises independently of the reproductive organ. Since a recent paper by Adams and Rae ('29) dealing with the function of the fat-body contains a comprehensive review of the literature concerning its origin, it is sufficient to state here that the majority of the evidence supports the contention that the fat-body is derived directly from a portion of the genital gland or has a common origin with it.

This study of the fat-bodies in Amblystoma maculatum was undertaken in the hope of obtaining not only information concerning their origin, but also a basis for an interpretation of their true significance.

The writer wishes to acknowledge at this time her great indebtedness to Prof. H. D. Reed not only for suggesting this problem, but also for his stimulating interest and helpful criticism during the entire course of the study. She is also grateful to Prof. S. H. Gage, of the Department of Histology and Embryology, for the loan of many excellent series of embryos and young larvae.

MATERIAL AND METHODS

The material consisted of a very complete series of stages of Amblystoma maculatum, ranging from cleavage to those beyond the completion of metamorphosis. Perenyi's, Zenker's, Gilson's, Flemming's, Bouin's, and aceto-formalin fluids were employed for fixation. Cedar oil proved to be the best clearer for the earlier stages. Xylene or toluene were satisfactory for all those above 20 mm. in length.

For the younger stages the entire animal was sectioned; in the older specimens only the trunk region was prepared. Numerous series were made of dissections which included the fat-bodies, the genital organs, the mesonephroi, and their mesenteries. Transverse, sagittal, and frontal series were available for all the early stages, and transverse and, in most cases, sagittal series for all the older ones. All sections were cut 7 to 10 μ in thickness and were mounted serially.

Of the many stains used, chloral hematoxylin, Ehrlich's acid hematoxylin, and Heidenhain's iron hematoxylin gave the best results. The latter was most effective in the older animals, giving excellent pictures both with and without a counterstain of eosin.

OBSERVATIONS

The very close series of material available for this study has made it possible to trace the development of the fat-body step by step. This organ makes its initial appearance relatively late in development, at a time when the gonad is clearly defined as a small organ consisting of germ cells interspersed with secondary or future sex-cord cells, the mass being surrounded by a peritoneal covering and the whole suspended in the coelom by a thin, double fold of peritoneum. The earliest sign of the fat-body was found in a 22-mm. larva. Burns' statement that this structure originates as a small bud of cells extending medially from the hilus of the gonad is confirmed. At first this tiny ridge, pushing out from the gonad, consists solely of some of those secondary, mesothelial cells which had previously found their way from the mesonephros into the reproductive organ and is of very necessity enclosed in a peritoneal sheath identical with that surrounding the gonad itself (figs. 1, 2, and 3, *F.B.*). This latter fact is of very considerable importance and its significance will be discussed later.

Both the gonad and the fat-body share a common blood supply furnished by a longitudinal vessel which extends lengthwise of each organ and is located in the dorsal juncture of the two (fig. 3, *B.V.*). The close connection between the chain of cells suspending the gonad and the fat-body in the coelom and the externa of the posterior vena cava is evident (figs. 2 and 4). This is a secondary connection, however, as the fat-body undoubtedly arises as a longitudinal outgrowth from the median side of the reproductive organ.

The appearance of the fat-body as an organ smaller than the gonad and containing only secondary cells is one of short duration. Transverse sections through the bodies of young larvae often show the fat-body and gonad to be practically identical in size and in structural appearance, since each may then possess numerous germ cells. Indeed, at a certain stage, so close may be the resemblance between these two organs that unless a person knew their relative positions in the body, he would be unable to distinguish them (fig. 6). This condition, namely, of the fat-body itself containing germ cells, is not difficult to observe and its common occurrence is without doubt of significance in an interpretation of the origin and fate of this peculiar organ.

Naturally such a situation suggests questions concerning possible explanations for the presence of reproductive cells in the fat-bodies. Have they migrated from the gonad into this organ or have they originated in the fat-body itself?

In all the material studied no evidence supporting a possible migration of germ cells was obtained. It should be stated, however, that in a few cases a propagative cell was found developing in the epithelium in that region where the reproductive gland and the fat-body unite, thus being in such a position as to make difficult, if not impossible, any postulation concerning its future site. Such a situation is pictured in figure 4, *G.C.* Is not this indicative of the potentiality of the peritoneum about these organs to form germ cells? On the other hand, the same material showed numerous germ cells, which might or might not be undergoing division, in such positions as to strongly suggest their origin from the peritoneum. They were seen in the fat-body in various situations, just below the epithelium, in the body of the organ, and often as far removed as possible from the gonad (figs. 5 and 6).

The presence of dividing germ cells in the fat-body is not by any means unusual. An excellent example of such a condition is shown in figure 5, *G.C.* Special attention is called to the location of this reproductive cell. It lies just below the epithelium and far removed from the genital organ. Practically the same location of a germ cell is represented in figure 6, *G.C.* This picture also shows the close resemblance which may obtain between the gonad and the fat-body.

An investigation of the origin of the germ cells in this same salamander has led the writer to believe that the germinal epithelium gives rise to the majority at least of the propagative cells. In this form all the successive stages in the transformation of a somatic cell into a reproductive cell appeared to be demonstrable. The early development of the fat-body as a bud from the gonad necessitates the same peritoneum surrounding both organs. Logically, therefore, the occurrence of germ cells, developing in the epithelium about the fat-body, is not to be unexpected. In the writer's opinion sufficient evidence in support of the origin of germ cells from the epithelium surrounding the fat-body appears to be available.

Late in June and onward, the fat-body commences to lose gradually any resemblance to the gonad and to take on the appearance characteristic of an adipose organ. At first small droplets of fatty substances may be seen in some of the cells, but these do not greatly alter the appearance of the organ (fig. 7, *F.G.*). Soon, however, the globules of fat increase greatly both in number and in size. Each large globule tends to fill and distend the cell in such a way that the nucleus is pushed to one side and any cytoplasmic structures are crowded to the periphery. At this time the fat-body appears filled with many immense globules of fat separated from one another by thin, irregular bands of material, and the whole organ, having increased tremendously in size, extends for the first time far beyond the caudal limits of the reproductive gland (figs. 8 and 9, *F.B.*). Associated with this deposition of fatty substances is the disappearance of all germ cells in the fat-body. In other words, the potentiality of the epithelium surrounding the fat-body to form germ cells is believed to be a temporary one.

In all the series of transforming larvae examined, including all animals killed during July and August, the fat-bodies were filled with adipose material. In a single individual whose metamorphosis was complete (an individual 50 mm. in length; killed August 5th) fat-globules were entirely absent.

DISCUSSION AND CONCLUSIONS

The presence of germ cells in the fat-body of certain amphibians has been described by several workers. Abramowicz ('13) mentioned finding reproductive cells in this body in Triton taeniatus (Schneid). She considered them aberrant cells. Witschi ('29) reported that in Rana sylvatica the developing gonadal ridge has three sections: the middle portion becomes the gonad proper, the anterior portion gives rise to the typical fat-body, and the posterior part often forms a small adipose body. He stated that, although the two fat-bodies are usually sterile, occasionally they contain single, scattered germ cells. On the other hand, Bouin said that in Rana temporaria, while both the genital gland and the fat-body have a common origin, the latter never possesses any propagative cells. In Amblystoma maculatum the fat-body is derived from a portion of the indifferent gonad and during its early developmental stages may contain numerous germ cells. The writer believes that the epithelium surrounding the fat-body is capable of forming these cells and that such a potentiality accounts for their presence.

Some workers have considered the fat-body to be a degenerate portion of the reproductive organ. In Amblystoma maculatum it possesses all the characteristics of the indifferent gonad until its body becomes distended with fat-globules. At this time all the germ cells disappear and no more are formed. In this sense only might it be considered a degenerate organ. Undoubtedly, the fat-body is not a degenerate structure in so far as it is a functional organ of apparently great importance in the metabolism of the individual at certain seasons of the year and in varying environmental conditions. Numerous investigations have given results supporting the nutritive function of the fat-body, its connection with development of the reproductive cells, and its seasonal variations (Ott, '24; Adams and Rae, '29, et al.).

From her own observations and without further study of this organ the writer limits herself to the following conclusions: the fat-body is distinctly of genital origin and for a certain time contains all the constituents of a gonad; it loses any gonadal resemblances early in ontogeny; and finally, its cells become so distended with large globules of fat that great increase in size results, the organ extending more and more into the coelom until it reaches far beyond the caudal limits of the reproductive organ.

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EXPLANATION OF PLATES

All photomicrographs, unless otherwise stated, are of transverse sections.

ABBREVIATIONS

B.V., blood vessel
F.B., fat-body
F.G., fat-globule
G., gonad
G.C., germ cell
G.E., germinal epithelium
Int., intestine
M., mesonephros

PLATE 1

EXPLANATION OF FIGURES

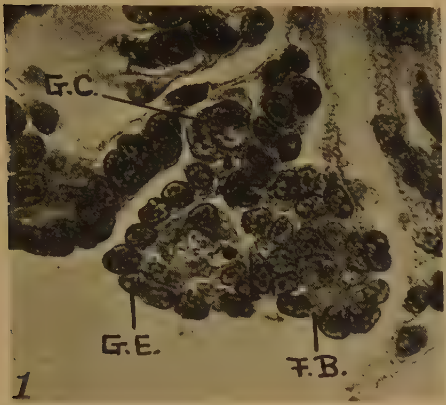
1 A photomicrograph of a section through the early fat-body and gonad of a 22-mm. larva. × 660. At this stage of development the fat-body, *F.B.*, and the gonad, *G.*, are nearly identical in shape and very similar in structure. A germ cell, *G.C.*, is shown developing in the germinal epithelium of the gonad.

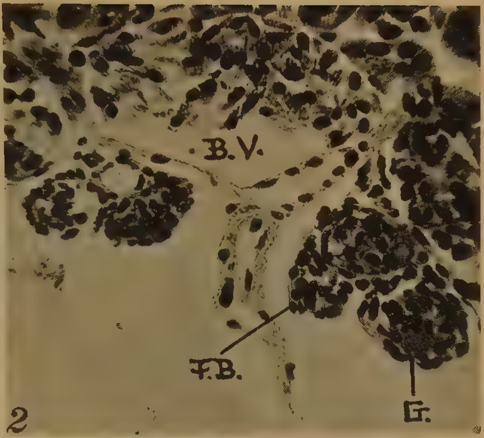
2 A photomicrograph of a section through the paired fat-bodies, *F.B.*, and gonads, *G.*, of a 36-mm. larva, to show the relative positions of these organs and their close connection with the posterior vena cava, *B.V.* × 264.

3 A photomicrograph of a section through the gonad, *G.*, and the early fat-body, *F.B.*, of a 36-mm. larva, to show especially the longitudinal blood vessel, *B.V.*, at the juncture of the two organs. × 660. The wall of this vessel is distended by the blood corpuscles. Compare this picture with figure 2.

4 A photomicrograph of a section through the fat-body, *F.B.*, and the gonad, *G.*, of a larva, 29 mm. in length. × 880. This picture shows an unmistakable germ cell, *G.C.*, in the epithelium midway between the gonad and the fat-body. Notice the uniform structure of the fat-body.

5 A photomicrograph of a section through the fat-body, *F.B.*, and gonad, *G.*, of a 37-mm. larva, especially to show the large, dividing germ cell, *G.C.*, in the fat-body. × 660. The germ cells in the gonad are also sharply defined. Attention is called to the larger size of the fat-body at this time.


G.C.
G.E.
F.B.
1


B.V.
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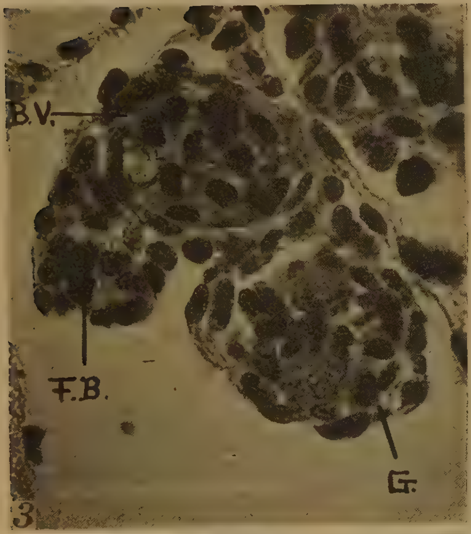
G.
2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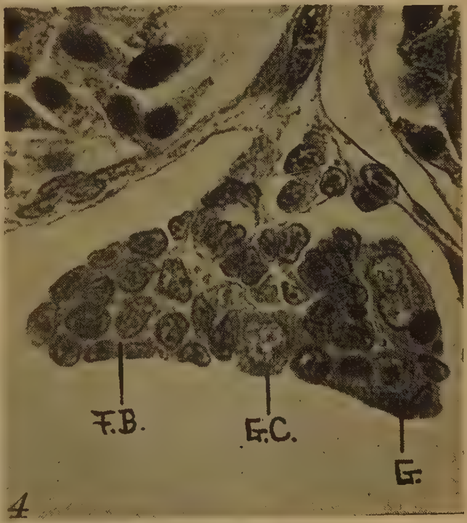
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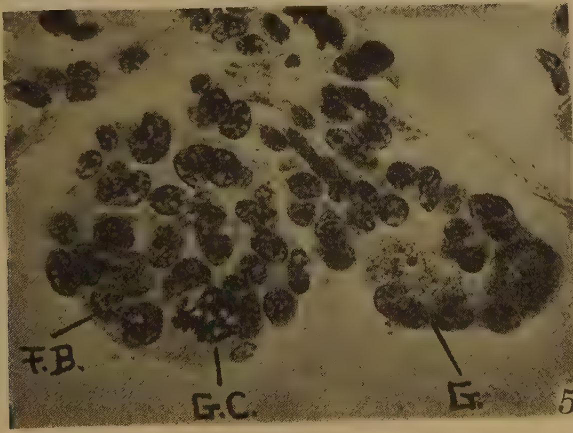

F.B.
G.C.
G.
5

PLATE 2

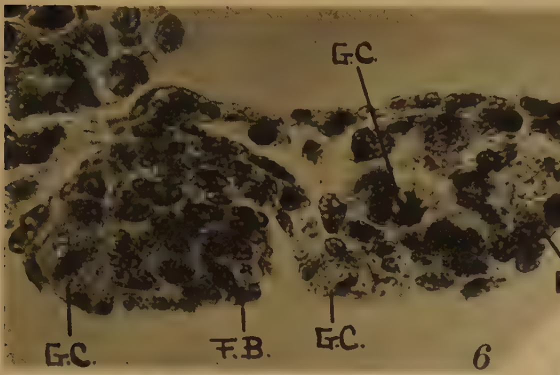
EXPLANATION OF FIGURES

6 A photomicrograph of a section through the fat-body and gonad of a 36-mm. larva. × 660. This shows, 1) a germ cell, *G.C.*, just below the epithelium of the fat-body, *F.B.*; 2) a germ cell differentiating in the germinal epithelium, and, 3) a germ cell in division in the gonad, *G.*

7 A photomicrograph of a sagittal section through a portion of the gonad and fat-body, *F.B.*, of a 45-mm. larva. × 264. At this time (animal killed July 3rd) a few small globules of fat, *F.G.*, are present and the nuclei of the fat-cells are very distinct.

8 A photomicrograph of a section through the fat-body, *F.B.*, of a 42-mm. larva in an early stage of transformation. × 150. This section is approximately through the middle region of the organ and shows it thickly packed with fatty substances (animal killed July 19th).

9 A photomicrograph of a section showing the fat-bodies, *F.B.*, the gonads, *G.*, mesonephroi, *M.*, and a portion of the intestine, *Int.*, of a 42-mm. larva. × 100. This picture shows that, even when the fat-body is well developed, in the caudal region it closely resembles the gonad and lacks any fat-globules (see caudal end of left fat-body).



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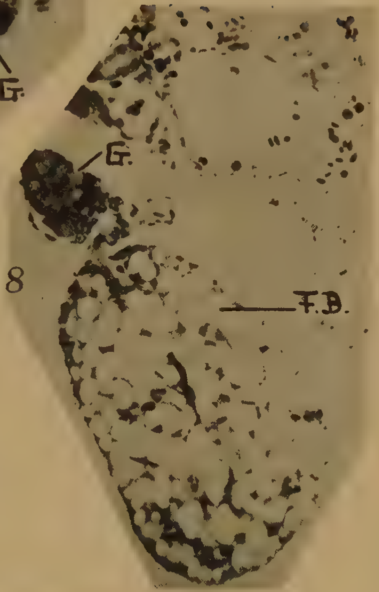

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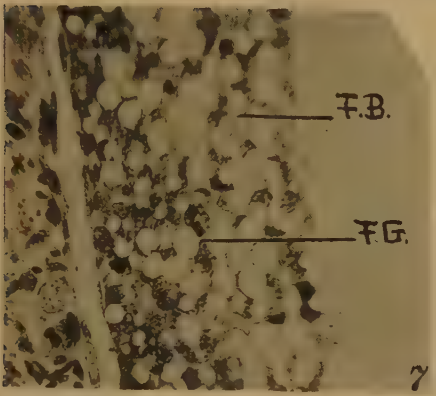

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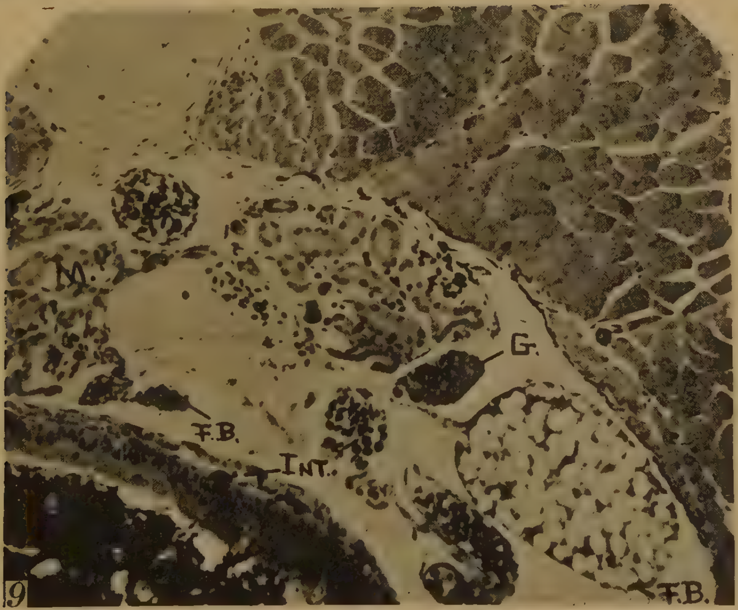

9

CAPILLAROSCOPIC APPEARANCE OF THE PULMONARY ALVEOLI IN THE LIVING DOG

D. M. OLKON AND MINAS JOANNIDES
Departments of Neurology and Surgery, Research and Educational Hospital, School of Medicine, University of Illinois, Chicago

ONE PLATE (TWO FIGURES)

The structure of the pulmonary alveolus and the capillary blood supply can be studied with a fair degree of accuracy in the living dog, through the transparent pleura. To study the functioning pulmonary alveolus by cutting into the lung tissue is not advisable, for many difficulties will arise, obscuring accurate observations. It is obvious that by cutting the lung we open the vessels and the air spaces, causing hemorrhage and leakage of air. Moreover, the lung, because of its elasticity, contracts rapidly as soon as the air escapes through the cut edges and the hemorrhage results in the filling of the alveoli with blood, so that the true structure of the alveolus is obliterated. By the use of the method described here, apparently an accurate picture of the alveolus may be obtained at varying degrees of lung distention.

A Zeiss skin microscope was used, the one devised by Ottfried Müller(1) giving a magnification of 60×. The advantage of this microscope over others is that it contains a small 6-volt lamp which is attached to the microscope. For stereoscopic view the Zeiss binocular stereoscopic microscope was used. Thus it is not necessary to project light from a distant point, nor is the constant adjustment of light necessary when a new field is observed. The 6-volt lamp is not hot enough to cause enough local elevation of temperature to dry the tissues. By the use of cedar oil, air was excluded and a much clearer field was thereby obtained.

TECHNIQUE

Dogs, frogs, and alligators were used in these experiments under ether anesthesia and positive-pressure artificial respiration. By means of a thoracotomy, the lung was exposed and observations were made at varying degrees of lung distention. The microscope was so placed over the lung that, as the organ moved with respiratory distention and collapse, the instrument also moved with the lung. No undue pressure was exerted at the point where the microscope was placed for observation. By constantly adjusting the focal distance, we were able to get an accurate view of the alveoli coincident with the respiratory movements.

OBSERVATIONS

When the lung is distended just enough to fill the chest cavity, it presents the following surface appearance. The alveolar sacs are of varying sizes and shapes. Each sac (fig. A) is composed of alveoli, which in turn are subdivided into one or more smaller areas (fig. B, *d 1*, *d 2*, and *d 3*) which assume variable shapes and sizes. The lines which separate these areas are not restricted to the surface, but represent shallow partitions within the alveolus, as determined by stereoscopic observation. What these septa-like processes are cannot be stated with certainty. But presumably, they represent networks of elastic fibers, such as have been described by histologists (compare Braus(2), vol. 2, fig. 109). That these septa-like partitions are present within the alveolus seems to be optically certain. Surrounding each alveolus is a more refractive zone made up of two walls, representing the walls of adjacent alveoli. What appears to be a large capillary (fig. B, *e*) is seen to occupy this space between the two walls, although presumably this is merely the capillary network seen on edge. From this large capillary many smaller capillaries, having the diameter of one red corpuscle, dip into the wall of each alveolus (fig. B, *f*, *j*, *k*, etc.). These smaller radially arranged capillaries do not end blindly, as suggested by the drawing, but anastomose everywhere with adjacent capillaries. The apparent fading out of the central portions is merely due to the increased pressure which produces the bulging of the walls at this point. (For a more detailed description of the alveolar capillaries and their significance, see companion article in Archives of Internal Medicine(3).)

Brief mention may be made here that the number of small capillaries visible in the alveolus varies with the amount of air pressure in the alveolus. That is, when the air in the alveolus is increased, fewer of these small capillaries are visible, and when the air is suddenly decreased in the alveolus, more of them become visible.

Another instructive feature of the capillary mechanism is an increase in the speed of movement of the blood cells in the larger capillary when the lung is excessively distended (fig. B, *e*). The smaller capillaries which take origin from this larger capillary (fig. B, *f, j*) and are seen inside the alveolus assume a deeper color and straighter outline (fig. B, *k*); and when the pressure is suddenly reduced, the flow in the larger capillary assumes a to-and-fro movement (fig. B, *j*), a-pause, and then resumes movement; the smaller ones also show changes in their color, caliber, and outline.

It may also be stated that even the smallest capillary appears to have the qualities of contraction and relaxation. In other words, a distinct tonus of capillary activity is clearly demonstrable. Moreover, when the pressure in the lung is increased, rupture of the smallest capillaries inside the alveolus may be seen (fig. B, *g*) with free erythrocytes in the air chamber.

With the changes of intrapulmonic pressure, the alveolar sacs and the alveoli vary in size. When the pressure is increased, the central portion of the alveolus becomes more prominent and appears as if stretched like a balloon (fig. B, *d 2*). The areas between the sacs become less prominent and at times compact. As the pressure decreases the alveoli become smaller (fig. B, *d 3*) and the interalveolar areas widen and again become more pronounced.

With normal respiration, as the alveolar sacs contract and expand we have noticed, at times, changes in the alveolar structure coincident with the movement of the alveolar air. For the air moves rather slowly, pushing its way into the contracted sac and distending it as it progresses. As the air bubble moves forward, each alveolus expands, assuming extensions and projections (infundibular form, *d 1*; and other shapes, as indicated in fig. B, *d 2* and *3*). The septa in the alveolus seem to determine the limits of the varying shapes produced by the changing air.

The pleura, on the other hand, appears to form only a loose covering over the lung. This may be assumed to be true, because we have often noticed that when the pressure in the lung was increased, air bullae were often seen to occur between the pleura and the lung. This occurred especially over areas where there was an oblique section of a small bronchus. We noticed that the loosest connection between the lung and the pleura is present at the junction of the parietal and visceral pleurae. It is undoubtedly at this point that mediastinal emphysema commonly finds its origin(4).

CONCLUSIONS

1. The alveolus in the living state shows novel appearances in that it is incompletely separated into compartments by shallow partitions, the latter presumably constituting the elastic-tissue network described by histologists.
2. These compartments or lesser air units assume a variety of shapes, form, and sizes under the pressure of inspiration.
3. Around each alveolus is what appears to be a single large capillary of the diameter of several red corpuscles, but presumably this is merely the capillary network of the alveolus seen on edge.
4. From this peripheral net many smaller capillaries are given off, which appear to dip into the wall of the smaller air units.
5. As the intrapulmonic air pressure is increased and the alveoli become distended with air, the inner portion becomes more prominent and its wall becomes more transparent. The

peripheral interalveolar space becomes less prominent and at times invisible.

6. With normal respiration, as the air moves from one portion of the alveolus to another, the compartments assume shapes similar to those of an oblong rubber toy balloon. As the air moves forward, the compartment contracts, assuming an infundibuliform appearance. It is perhaps by this means that the air is trapped in the alveolus, thus insuring sufficient time for the exchange of gases between the blood and alveolar air.

7. The normal pleura may be considered as a transparent window through which changes in the structure and function of the alveolus can be observed.

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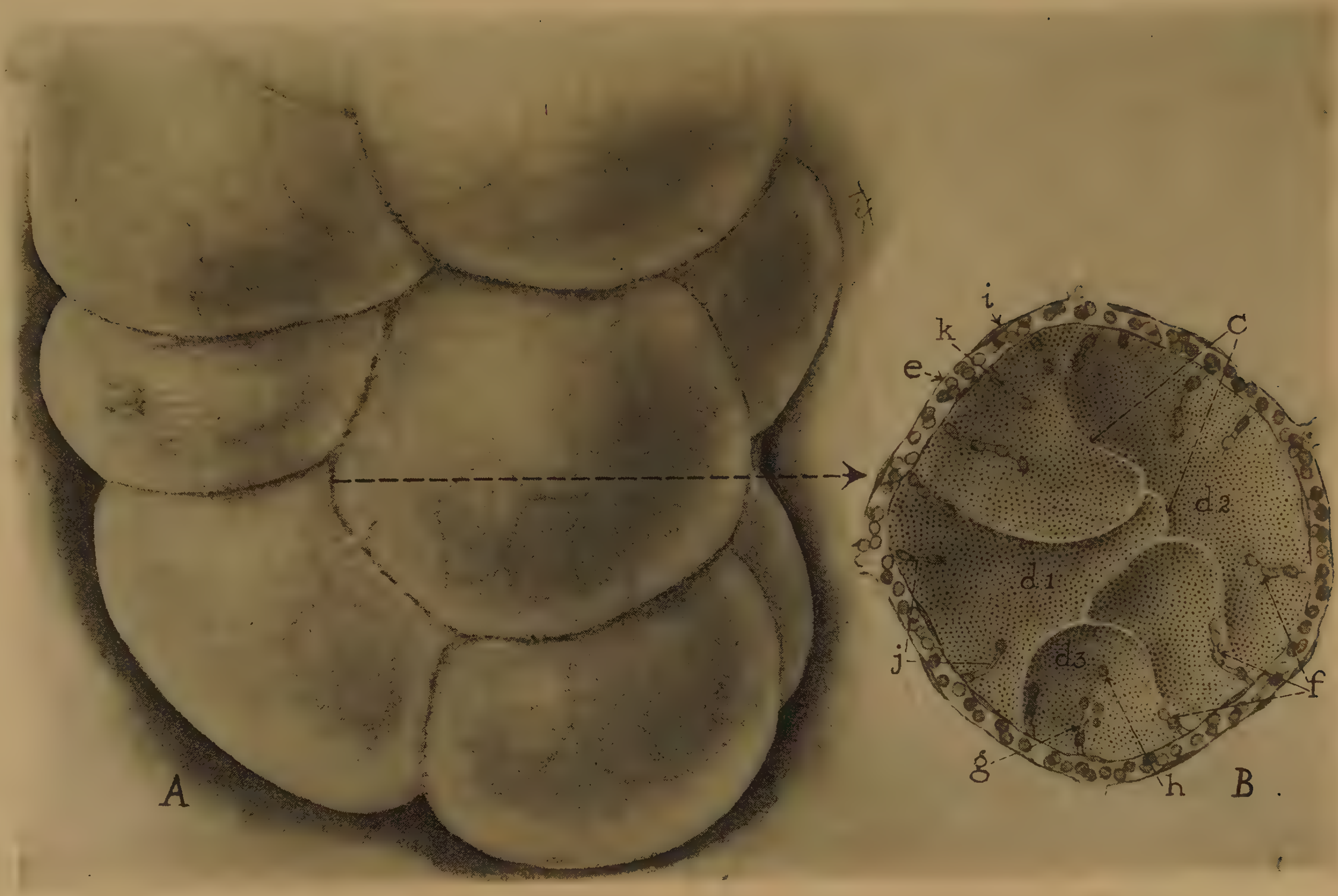
PLATE 1

EXPLANATION OF FIGURES

A Diagram of surface appearance of an alveolar sac in the living dog.

B Single alveolus. *c*, partitions within alveolus; *d 1, 2, 3*, subsidiary air spaces; *e*, large capillary net surrounding alveolus; *f*, smallest capillaries; *g*, ruptured capillary; *h*, free-floating red cell; *i*, endothelial nucleus; *j*, contracted capillary; *k*, straight capillaries.


A
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C
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AN ANOMALOUS INDIAN OCCIPUT [1]

WILLIAM F. HOHEISEL

ONE PLATE

INTRODUCTION

Dickson's mound-builders' tombs, Lewistown, Illinois, afford a study of unusually well-preserved, entire Indian skeletons, aggregating at present over 250. They represent only a small cross-section of the entire mound, which for centuries was used by our American aborigine as a burial ground.

Through the courtesy and cooperation of Dr. D. F. Dickson, the author has begun an extensive field study of normal and pathological variations in Indian bones. Much credit is due Doctor Dickson for the character of the excavations and his scientific interest. As anatomical data are collected, the results should become increasingly important anthropologically.

The anomalous skull to be described is of peculiar anatomical interest, and it assumes priority only because of its rarity in the collection. It shows the following anomalies: 1) a partially persistent interparietal; 2) two accessory interparietals; and, 3) four unusually large wormian bones.

EMBRYOLOGY

Extensive embryological studies have proved that normally the squamous portion of the occipital bone develops from four ossification centers which appear at about the eighth week. The lower pair of centers develops from cartilage into the supra-occipital, while the upper pair is deposited in membrane and develops into the interparietal. The two pair are fused in the median plane at the end of the third month. Laterally, however, a fissure (the sutura transversa) persists until after birth. In the adult the horizontal line of union of the supra-occipital and interparietal is called the linea suprema. Although not always prominent, it describes an arc with a superior convex border. In a normal skull the linea suprema is found directly above the external occipital protuberance and the superior nuchal line.

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[1] Contribution from the Zoölogical Laboratory of the University of Illinois, no. 367.

Occasionally, two additional centers of ossification make their appearance somewhat lateral to those mentioned above, though more commonly they occur superior to the interparietals. They may grow downward in the interspace between the two halves of the interparietal. These accessory centers are classified as preinterparietalia by Stieda(2), Chiarugi, and Terry(4). The term accessory interparietals is used interchangeably with the above. It has been demonstrated that these accessory bone centers, when present, develop at a later stage than the interparietalia and are usually associated with the apex of that segment. When fully grown they are three-sided, and, when fused along the midline, form a diamond-shaped figure. Usually they fuse solidly with the rest of the interparietal, although a ridge of bone may indicate the original line of fusion.

ADULT SKELETAL FEATURES OF THE PART INVOLVED

The following skeletal features are involved in the squamous portion of the occipital bone:

The squamous portion of the occipital bone (Piersol, 1) forms the lower and back part of the skull. Below it contributes the posterior boundary of the foramen magnum and joins the exoccipitals. The lateral borders meet above at a sharp angle. These borders may be subdivided into a lower part (ending as the projecting jugular process) which ascends nearly vertical in articulation with the mastoid part of the temporal, and a higher part, very serrated, and joining the parietal. The bone is angular at the junction of these two divisions.

According to Terry, this is the lateral angle. Quoting Terry(4):

> The squamous part (supraoccipital and interparietal) presents on its convex posterior surface, and midway between the superior angle and the posterior margin of the foramen magnum, a prominent tubercle known as the external occipital protuberance. From this landmark a vertical ridge runs down to the foramen magnum. Arching lateralward from the external occipital protuberance toward the lateral angle of the bone is a semicircular ridge, the superior nuchal line. This divides the surface into two parts, an upper planum occipitale and a lower planum nuchale. Above this line is a second, less distinctly marked ridge, the highest nuchal line or *linea suprema.* The lower part of the surface is uneven and subdivided into an upper and lower area by the *inferior nuchal line.*

Owing to a diseased condition of the skull under consideration, some of the landmarks just enumerated are indistinct. The conditions found in the occiput will be described, starting with the posterior-inferior surface.

DESCRIPTION AND CLASSIFICATION OF THE ANOMALY

That part of the supra-occipital forming the posterior boundary of the foramen magnum is missing. The parts immediately superior and lateral to it are normal, and it may be assumed that the missing portion likewise was normal. The inferior nuchal line is on the missing part, hence it is not available for identification. Disease seems to have accentuated the superior nuchal line (fig. 1, *n*) in certain regions and to have weakened it in others. The occipital protuberance (*p*) has been reduced to a level internal to that of the superior nuchal line, but it is still palpable and can be felt more readily because of an abnormal fossa (*f*) of considerable size lying immediately superior to it. This fossa, no doubt, is the result of absorption of bone due to pressure atrophy caused by a scalp infection.

The linea suprema (*s*) is hardly distinguishable except on palpation, and were it not for a persistent remnant of the sutura transversa (*t*) on the right side, one might identify as this landmark an anomalous suture (*a*), which is convex superiorly, and about 3 cm. above the external occipital protuberance. It is true, however, that the position of the groove for the transverse sinus (found on the anterior surface) is identical with that of the linea suprema. Examination of a series of Indian skulls has disclosed this correlation and simplified the diagnosis of this bony marking. Without the above correlation, one might have assumed that the crescent-shaped bone (*ip*) above the superior nuchal line (really the interparietal) was an extension of the supra-occipital, and its superior border, the linea suprema. The greatest width of this bone area is about 3 cm. I know of no recorded supra-occipital extending so far superior. One can conclude, therefore, that the superior limit of growth of the interparietal in this case is the convex suture (*a*) mentioned as defining an arc with its highest point about 3 cm. above the occipital protuberance. It will also represent the basal limits of two large, isolated, asymmetrical bones (*pi 1, pi 2*) which complete the squamous portion. It will be seen that they occupy the major portion of the area normally covered by the fused interparietal. These anomalous bones cannot be classed as wormian in character. From their position, size, and regular contours I conclude (on the basis of somewhat similar cases recorded) that they represent the third or accessory pair of ossification centers, the preinterparietalia distinguished by Steida(2).

An examination of the skull (fig. 1) discloses that the right preinterparietal (*pi 2*) encroaches on the left (*pi 1*) for some little distance beyond the midline, and consequently is much the larger of the two. It also forms the superior angle (*an*) of the squamous occipital in conjunction with two unusually large wormian bones (*w 1, w 2*), the lower one of which (*w 2*) definitely forms the apex of the angle.

On the left side, in the region of the asterion, another large wormian (*w 3*) is isolated. It measures roughly 14 × 22 mm.

In a corresponding position on the right side, an accessory bone (*w 4*) measures about 17 × 30 mm. Both are inclosed in a space between the parietomastoid and occipitomastoid sutures. Apparently there is no previous record of such a variation in American Indian crania—certainly not in the Illinois group. Neither has the occurrence of lambdoidal suturals been previously recorded for Illinois (Sullivan, 3).

Inasmuch as the history of development is so indelibly written in this skull because of the indicated presence of original centers of ossification and the usual diagnostic landmarks of bone, it is not difficult to classify the two bones in the lambdoidal suture and those between the occipitomastoid and parietomastoid sutures as wormian.

It can be concluded that this mound-builder Indian skull represents several anomalous conditions: 1) a partially persistent interparietal; 2) complete isolation of an accessory pair of ossification centers, the preinterparietalia or accessory interparietals, which are bilateral and asymmetrical; and, 3) four unusually large wormian bones, which may be classified primarily on the basis of position and the fact that all other centers are referable to the occipital bone itself.

FREQUENCY

Bianchi, in a study of sixty-five fetuses of from four and a half to nine months' term, found four cases of the preinterparietal. Later, the same author found six out of twenty-three embryos showing the same condition. These were both bilateral and unilateral: about 50 per cent representing each condition. Very obviously, they would include many asymmetrical pairs as a result of unequal growth of the individual centers. Again, sometimes the right would be persistent and at other times the left. Marimo states that he found the preinterparietal in 3.3 per cent, Chiarugi finds it in 4.6 per cent, and Steida in 3.3 per cent, which he bases on an examination of 669 skulls, in which he found twenty-one showing the isolated preinterparietals.

The interparietal has been found isolated frequently in adult Peruvian skulls. In this racial stock, according to Steida(2), the percentage is as high as 5.46 per cent—the highest recorded for any race. Sullivan(3), a modern worker, has shown that many other groups have a higher frequency. An index of this kind can be of definite anthropological significance only when applied on a large scale to the primitive Americans, the mound builders. Rather, the normal variations of the skeleton should figure strongly in many new and true race indices.

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PLATE 1

EXPLANATION OF FIGURE

Posterior view of the Indian cranium, × 1.

ABBREVIATIONS

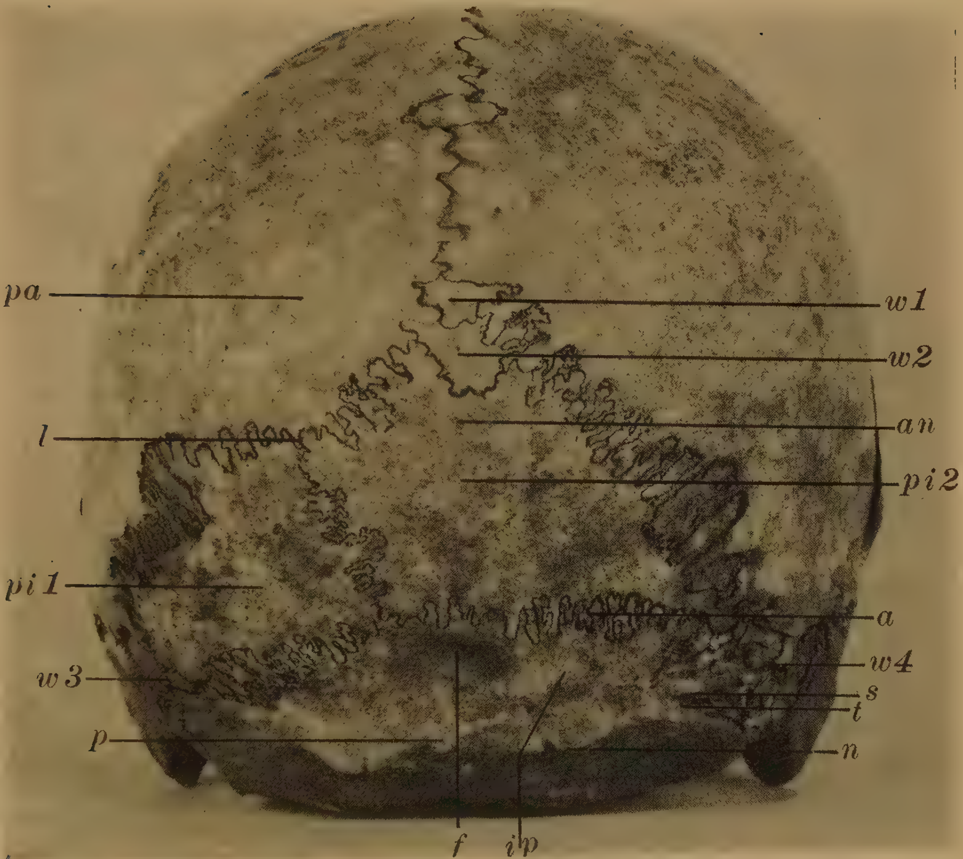
a, anomalous suture
an, superior angle
f, abnormal postcranial fossa
ip, crescent-shaped interparietal
l, lambdoidal suture
n, superior nuchal line
p, occipital protuberance
pa, parietal bone
pi 1, left preinterparietal (accessory interparietal)
pi 2, right preinterparietal (accessory interparietal)
s, linea suprema
t, sutura transversa

Wormian bones of superior angle (lambdoidal suturals):

w 1, superior wormian
w 2, inferior wormian

Wormian bones between occipitomastoid and parietomastoid sutures:

w 3, left asterion wormian
w 4, right asterion wormian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STUDIES ON THE VACUOME AND THE GOLGI APPARATUS IN THE ACINAR CELLS OF THE PANCREAS OF THE RAT

H. W. BEAMS
Laboratory of Histology and Embryology, Medical School, University of Virginia

ONE PLATE (SIXTEEN FIGURES)

At the present time probably more attention is being paid to the Golgi apparatus than to any one of the other cellular components. The Golgi apparatus was first discovered by Golgi in 1898, as a new and independent cell organ universally present in animal cells, and since that time many contributions on its structure and function have been made. Notwithstanding all these studies, however, it remains to date a subject of confusion and decided controversy. Recently, the problem of the Golgi apparatus, its structure and functional significance, has been discussed at length in a number of scholarly papers (Bowen, '26, '27, '28, '29; Parat, '28; Guilliermond, '27; Nassonov, '26; Owens and Bensley, '29, and others), to which the reader is referred for a detailed review of the subject and for an extensive bibliography.

Briefly, two outstanding current conceptions concerning the nature of the Golgi apparatus may be summarized as follows: First, that which may be designated as the modified theory of Golgi, namely, that the Golgi apparatus is a new and independent cell organ universally present in all animal cells (and perhaps plant cells—Bowen, '28) in a great variety of forms, and possessing a definite structure, probably in the nature of a lipoid, which is blackened by prolonged exposure to silver nitrate and osmic acid (Cajal, Nassonov, Bowen, Gatenby, Ludford, and many others). The alternative view, which seems to have been first suggested by Bensley and recently urged by Parat, Guilliermond, and their associates, holds that the Golgi apparatus results from the reduction of osmic acid and silver nitrate within, around, and between vacuoles and granules in the cytoplasm of the cells producing the structures described by many workers as definite cytoplasmic components. Furthermore, it is held that these vacuoles and granules can be stained vitally and selectively with neutral red and later may be seen to align and fuse to form the typical (factitious) net-like appearance of the Golgi apparatus. The classical Golgi apparatus is accordingly considered by these investigators as an artifact; the reality they claim is the vacuome.

My interest in this problem was greatly stimulated as a result of our recent failure to find the Golgi material in the salivary glands of the Chironomus larvae (Beams and Goldsmith, unpublished), as described by Parat and Painlévé ('24). Instead, our observations are in harmony with those of the recent findings of Krjukowa ('29), namely, that the Golgi material in the Chironomus larvae exists as discrete bodies which are never observed to fuse and form the network as described by Parat and Painlévé ('24, '25, '28). This case is cited as of especial importance, because it was upon observations on the salivary glands of the Chironomus larvae that Parat chiefly based his vacuome theory. Accordingly, this material remains his classical illustration ('25, '28).

The pancreas was selected for this investigation because it has been the object of numerous studies on the Golgi apparatus since the time of Negri ('00), only two years following the discovery of the 'apparato reticolare interno' by Golgi in 1898. Since that time the Golgi apparatus has been studied in the pancreas by v. Bergen ('04), Kolster ('13), Kolmer ('16), Bensley ('11), Cajal ('14), Nassonov ('23, '24), Cowdry ('22, '25), Bowen ('26), and others. These students of the Golgi apparatus are in remarkable agreement that the material exists in the acinar cells of the pancreas in a typical reticulated condition, occupying a position between the nucleus and the lumen of the gland. This network seems to be subject to some variation and fragmentation (Nassonov, '24) during different physiological conditions of the cell.

The pancreas has likewise furnished the material for fruitful investigations in the support of the vacuome theory. Parat and Painlévé ('25) noticed that the vacuome in the acinar cells of the pancreas appears, following treatment by neutral red, as small, medium, and large vacuoles situated in the cytoplasm in the neighborhood of the distal pole of the nucleus. The young zymogen granules also were colored by the neutral red, varying in shade from a deep red to an orange, depending primarily on the degree of their maturation. The vacuoles appear to coalesce and to extend around and among the zymogen granules, simulating a network and forming 'croissants,' which Parat likens to the 'mailles' of the network described by Nassonov. He further contends that the vacuome is the source of new zymogen formation, presumably by a condensation of the vacuoles. In a later communication, Parat ('28) elaborates his views and figures in color the acinar cells of the pancreas of certain amphibians following treatment with neutral red and Janus green. Many of the zymogen granules that have extended beyond the vacuome region are seen to possess 'croissants' not unlike the osmiophilic caps figured by Bowen ('24) on the zymogen granules of Cryptobranchus. Parat and Painlévé have published pictures, after employing osmic-acid and silver-nitrate methods, which were similar to those secured by intravitam staining with neutral red. They also saw a difference in the volume of the vacuome depending on the different physiological conditions of the cell.

Covell ('28), in a microscopical study of pancreatic secretion after the injection of neutral red into the living animal (mouse) previous to and following the injection of pilocarpin, directly observed the formation of vacuoles and in part the formation of zymogen granules. He was able to follow both zymogen granules and the neutral-red granules throughout their course in the cell. The neutral-red-stainable granules appeared for the most part in the area of the cell basal to the zymogen granules, extending well along either side of the nucleus. In discussing the events which took place while the cell was under continuous observation, Covell says:

> The zymogen granules were never seen to arise de-novo, and no transition could be found in this experiment, nor in many others like it, between the brilliantly colored neutral-red granules [vacuome] and vacuoles and the zymogen. The neutral-red-stained granules are smaller than the zymogen granules, but when, as is often the case, they are embedded in red-tinted vacuoles the discrepancy in size is not so marked. Zymogen granules were never seen to be lightly colored by the stain. Yet the neutral-red granules and vacuoles are certainly grouped in the intermediate zone of the cell in which zymogen formation must be actively taking place.

Covell accepts the neutral-red granules as the Golgi apparatus and concludes that the vacuome has no relation to the formation of zymogen granules.

Ma and Chang ('28) have recently stained the Golgi apparatus in a number of different tissues (including pancreas), by injecting a saturated solution of neutral red alone and a mixture of neutral red and sudan III. Following this technique, they find that the Golgi apparatus appears in the form of a network, or as rods and granules of varying sizes. They have checked the location, shape, and structure of the Golgi apparatus in the living cell with that of the metallic-impregnation methods and find that the neutral-red preparations invariably give as clear a picture of the Golgi apparatus as do those of the older silver and osmic-acid methods.

It is a pleasure to record here my grateful appreciation to Prof. H. E. Jordan for his interest, kind criticisms, and helpful suggestions given me during the course of this study.

MATERIAL AND METHOD

Adult rats were injected intraperitoneally or subcutaneously with a saturated solution of neutral red in distilled water or normal saline. They received 4 cc. of a saturated solution of neutral red at two-hour intervals until 8 to 12 cc. of the dye had been injected. After three to six hours, the animals generally turned a pink color and their urine became a deep red. The rats were usually killed six to eight hours after the first injection. A thin piece of the pancreas was removed from the animal and placed on a slide with a few drops of Ringer's solution, and the edges of the cover-glass sealed with vaselin. Preparations of this type may be studied several hours before a noticeable effect of cytolysis is apparent. With this technique the zymogen granules should appear a clear color, the cytoplasm in some cases slightly pink, and the neutral-red granules and vacuoles a deep pink or red. Smaller injections and shorter intervals between the first injection and the examination of the pancreas result in a less voluminous vacuome and one of lighter color.

After the animals have been injected with the neutral red and a portion of their pancreas revealed clear red bodies, small pieces were placed in Mann's solution for from fourteen to eighteen hours, after which they were washed in distilled water for one-half to three hours, and then placed in clean glass-stoppered bottles containing 2 per cent osmic acid. The vials were then put in an incubator at a temperature of from 35° to 37°C., from three to six or eight days, the method of Nassonov ('23) being used to determine when suitable impregnation has occurred. Following the above procedure, the red-stainable granules are blackened and the Golgi apparatus makes its appearance typically as a network. Portions of the pancreas which have received neutral-red injections may be mounted over a slide containing a well with 2 per cent osmic acid, and the granules can then be observed to change from a red color to a more or less clear or light brown and finally to a deep black.

DESCRIPTION

The Golgi apparatus in the acinar cells of the pancreas of the rat agrees substantially with the observations of Nassonov ('24) and Bowen ('26) on the Golgi apparatus in the acinar cells of the pancreas of the mouse and cat, respectively. The general location of the apparatus seems always to be in the area between the nucleus and the lumen of the gland. The Golgi apparatus appears typically as a network, although in some cases a discontinuous reticulum may occur. Bowen ('26) has suggested that the discontinuous reticulum is probably due to faulty technique. The question of the finer morphology of the reticulum has been discussed by Bowen ('26). He concludes that the thread-like appearance of the network is perhaps much more complicated than its simple superficial appearance would indicate. It has been pointed out by Nassonov and Bowen that in many cases the strands of the Golgi apparatus are probably plate-like; this would readily account for much of the variation in appearance. Also, the strength of the impregnating media and the time of exposure to the impregnating solution seem likewise to play an important rôle in the production of variations in the appearance of the reticulum. In very early stages of the impregnation of the Golgi apparatus (fig. 9) only the very fine outlines of the reticulum are discernible. As impregnation progresses the strands become much more robust and prominent. When the impregnation has been intense, the strands usually appear dense and massive, and the finer details of the reticulum are not apparent. When the impregnation has taken place perfectly, the strands appear sharp, and their details are no longer exactly uniform in character (fig. 1).

Variations in the distribution of the network are also quite frequent in the pancreas. In certain cases (fig. 2) the strands of the reticulum may extend well throughout the mass of zymogen granules in the direction of the lumen. In other preparations the Golgi apparatus may consist of a more or less complicated condensed reticulum, occupying its characteristic position in the Golgi zone near the apical pole of the nucleus (figs. 1, 14). All gradations between these two conditions may be observed in different cells of the same preparations. The Golgi apparatus in the acinar cells of the pancreas is obviously subject to considerable variation. The findings of Nassonov ('24), following the injection of pilocarpin, suggest that different physiological conditions of the cells have a marked influence on the appearance of the Golgi apparatus. Recent observations by Ludford and Cramer ('27), Weatherford ('29), and others would tend to bear out this interpretation. It is of especial importance to note here that no such structures (vacuome) as described by Parat and Painlévé, and by Covell, following intravitam staining by neutral red, could be found after modified Mann-Kopsch technique, unless the preparations are previously intravitally stained by neutral red. This is corroborated by practically all previous studies on the Golgi apparatus in the acinar cells of the pancreas following metallic-impregnation methods.

The vacuome of the acinar cells of the pancreas following the injection of neutral red into the living animal

After the injection of neutral red subcutaneously or into the peritoneal cavity of the rat, certain bodies in the acinar cells of the pancreas seem to have a definite affinity for the dye. These bodies have been recently designated as the vacuome and are thought by some to be a universal occurrence in all plant and animal cells. In the pancreas the vacuolar area seems to be on the boundary line between the basal zone of the cell and the zone of zymogen granules, occupying a position between the nucleus and the luminal end of the cell (figs. 3, 4, 5). However, in some instances the neutral-red bodies may be found scattered among the zymogen granules and extending well along the sides of the nucleus in the basal zone of the cell. The color of the vacuome, following the intravitam staining by neutral red, appears to vary from an orange to a deep red, depending probably on the pH and the intensity of the staining reaction. A considerable variation in size is also characteristic of these bodies. Some of the neutral-red bodies are slightly larger in volume than the mature zymogen granules, while others seem to be somewhat smaller.

There seems to be some inconsistency in the terminology used to designate the neutral-red bodies in the pancreatic cells, all agreeing, however, that they belong to the new realm of structures designated as the vacuome. In an abstract I used the terminology of Parat, which describes these neutral-red bodies as vacuoles (Beams, '29), while, on the other hand, they have been recently described by Covell ('28) as granules. From the morphological point of view these bodies appear for the most part as granules, although it is not uncommon to find one or more granules embedded in a lightly red-tinted vacuole (fig. 7), as observed by Covell.

Parat and Painlévé ('25) observed the coloring of the immature zymogen granules by neutral red, which, according to their theory, if I interpret it correctly, is the product of a condensation of the vacuoles. The coloring of the young zymogen granules as described by Parat seems to find support in the observations of Bensley ('11). Covell ('28), on the other hand, observing the neutral-red and zymogen granules throughout their course in the cells of the living animals, failed to observe any suggestion whatever that the neutral-red granules bear any relation to the zymogen granules. The findings presented in this paper corroborate those of Covell, that the zymogen granules are very rarely, if ever, tinted by the dye. Also, the fact that neutral-red granules similar to those in the pancreas have been found in the nerve cells (Covell and Scott, '28) makes it very evident that these granules are not confined to the acinar cells of the pancreas. The cytoplasm of the cell may be tinted a light pink in some instances when the vacuome is intensely stained by the neutral red. Especially is this true in the zone of zymogen granules.

There is considerable variation in the general volume of the vacuome present in the acinar cells (figs. 3, 5). This has been described by Parat as a result of different physiological conditions of the cell. Other factors which appear to affect the size of the vacuome are the amount and concentration of the dye administered and the time it is permitted to act before the tissue is examined. In cases where a voluminous vacuome is present in the cells, certain of the neutral-red bodies lie closely adjacent, and have been described by Parat as coalescing to form an indefinite mass simulating in certain instances a network. In my studies I have never observed any extensive fusion of the vacuoles as described by Parat. Occasionally, one or more adjacent vacuoles give the appearance of fusion, but these conditions are rare and seem to be an exception rather than a rule. In many cases (figs. 5, 6) the neutral-red bodies can be observed among the zymogen granules isolated some distance from the vacuolar zone. They possess in some instances a definite alignment, which has also been figured by Covell (fig. 6). These neutral-red bodies do not usually form 'croissants' on the zymogen granules, but retain their individuality, all of which points strongly to the assumption that there is no direct morphological relation between the zymogen granules and the neutral-red bodies. Furthermore, no gradations are apparent between the neutral-red bodies and the young zymogen granules, as would undoubtedly be the case should the young zymogen granules be colored by the dye.

Intravitam-stained vacuome followed by osmic-acid impregnation

A phenomenon of great significance is that observed by Covell and Scott ('28), Dawson ('28), and others, that the vitally stained vacuome will, upon the application of osmic acid, osmic-acid fumes, or fixatives containing osmic acid, turn black. This has been found to be the case in the acinar cells of the pancreas. If a portion of intravitam-stained pancreas be fixed and impregnated by the osmic-acid method above described, the vitally stained neutral-red bodies will retain their identity and assume a deep black color (fig. 8). This process has been observed directly under the microscope. The typical procedure seems to be that described by

Covell and Scott ('28). At first the vacuoles and granules become more or less clear or light brown and later turn a deep black. No noticeable change in position or tendency toward alignment and fusion of the neutral-red bodies was observed. It will be recalled that in some cases the neutral-red bodies in the vitally stained preparations lie closely adjacent, and in certain instances a fusion of one or more vacuoles seems to have taken place. The same appearance may be observed in clearly impregnated neutral-red osmic-acid preparations. It must be emphasized, however, that this is a rare occurrence in successfully impregnated preparations and does not represent the usual process.

Tissues in which the neutral-red bodies have been deeply stained, and subsequently overimpregnated by osmic acid, may give almost any sort of appearance in the cells. Such preparations contain artifacts of varying degrees of osmic-acid reduction in the cytoplasm. This is especially striking in the cells which are near the center of the sections. Figure 15 represents a typical case of a cell that has been deeply stained by neutral red and subsequently exposed for a long time to the action of osmic acid. The osmic acid has become reduced in the cytoplasm, and a massive appearance of what seems to be a network is seen occupying the major part of the cell. In fact, in some instances the whole cell is darkened to such an extent that the zymogen granules are completely obscured. I have not been able to satisfy myself as to whether or not the osmic acid has become reduced in the cytoplasm between the neutral-red bodies, simulating this massive reticulum as is sometimes observed, or whether it really represents an actual fusion of the neutral-red osmiophilic bodies, or both. In any case it is obviously an artifact; and in cells vitally stained with neutral red and subsequently properly fixed and impregnated by osmic acid, the vacuome appears distinctly as discrete bodies. The Golgi apparatus takes the form of a typical network composed of sharp darkly impregnated strands among the clear and light brown zymogen granules. It seems probable, therefore, that in the most favorable preparations, the action of the osmic acid on the neutral-red bodies causes very little if any change in their general morphology.

Successful preparations of the acinar cells of the pancreas which have been intravitally stained with neutral red, followed by the modified Mann-Kopsch-Weigl technique, demonstrate the vacuome as a structure resembling in all its details the vitally stained vacuome. Besides the vacuome, if the preparations are successful, a typical Golgi apparatus makes its appearance in the cells (figs. 10, 11, 12, 13, 14). The vacuome may be localized in the vacuolar area, as previously described at the apical pole of the nucleus—the same general region which may also be shared by the Golgi apparatus (fig. 10). In some cases where the Golgi apparatus and vacuome are localized at the apical pole of the nucleus, the Golgi strands can be seen extending between and around the deeply impregnated vacuome (fig. 10, 11), but in no case where a clear and sharp impregnation has taken place is there any evidence that the vacuome is ever fused with the Golgi apparatus. In other instances where the strands of the Golgi apparatus have extended well throughout the area of the zymogen granules in the direction of the lumen of the gland, the vacuome can be seen scattered among the granules (figs. 12, 13). The position of the neutral-red bodies seems to be affected somewhat by the strands of the Golgi apparatus, as their position is usually revealed along the side of the clearly impregnated Golgi strands. The elements of the vacuome, when scattered among the zymogen granules, seem to retain their more or less spherical form, and rarely do they appear to form 'croissants' as described by Parat.

Oftentimes the Golgi apparatus and the neutral-red bodies are seen to occupy distinctly separate positions in the cell. Figure 14 represents a cell in which the vacuome is well impregnated and localized at the apical pole of the nucleus at the junction of the basal zone of the cell and the zone of zymogen granules. A distinct sharply impregnated network of the Golgi apparatus can also be seen in the cytoplasm between the zymogen granules some distance from the vacuome. The general appearance of the vacuome and the Golgi apparatus following the neutral-red osmic-acid technique resembles in every respect the usual appearance of these elements following neutral red and osmic acid, respectively. In other words, they appear as discrete structures and no evidence accrues that the classical Golgi apparatus might have been formed as the result of the action of osmic acid on the neutral-red bodies.

Here, as in the neutral-red preparations previously described, the individual neutral-red osmiophilic bodies vary considerably in size and intensity of impregnation. The majority of the neutral-red osmiophilic bodies appear as homogeneous black granules of spheroidal or angular form. This is not invariably the case, however, as frequently a vacuole with a dark boundary and a granular or clear center may be observed. I have not been able to follow clearly in these preparations the vacuoles which are sometimes encountered surrounding the neutral-red granules in the intravitam-stained tissue. It is apparent that the vacuome is composed of a more or less heterogeneous group of structures all of which are probably colored by neutral red and subsequently impregnated by osmic acid.

Following intravitam staining with neutral red and subsequent treatment by osmic-acid impregnation, the zymogen granules present a clear brown color. Even the very small (immature) zymogen granules give the same appearance as do the mature zymogen granules. It is probably true, as pointed out by Covell, that the formation of new zymogen granules takes place in the vacuolar zone of the cell, although there is no obvious relationship between the zymogen granules and neutral-red osmiophilic bodies. Consequently, the results of this study confirm the conclusions of Covell ('28) that the zymogen granules are never colored by neutral red following its injection into the living animal.

DISCUSSION

The results of this investigation support the conclusions that the classical Golgi apparatus and the vacuome are two distinct substances in the acinar cells of the pancreas. Incidentally, this raises a serious objection to the vacuome theory in general, which interprets the Golgi apparatus as a factitious deposition of the impregnating medium within, in the walls and interspaces of the vacuome.

Bensley ('10) seems to have been the first who definitely advanced the idea of the vacuome (of Dangeard). He considered the vacuome of animals and plants homologous structures. He holds to the canalicular type of apparatus, while Parat and Painlévé's intravitam studies with neutral red demonstrate that the vacuome consists for the most part of isolated vacuoles. Parat observed also that at times the vacuoles appear to coalesce to form canals (Chironomus larvae) at the moment of excretion, while in other cases the vacuoles are artificially fused, due to the action of the impregnating media. To account for the discrepant observations of a canalicular type of apparatus and one consisting of a collection of isolated vacuoles, Bensley ('29) points out that a slight change in surface tension might easily convert a row of vacuoles into a canal or a canal into a row of vacuoles. In order to assume this view, it must be granted that the canals and vacuoles are fluid, in which case it would appear likely that if a row of vacuoles fuse, they would tend to retain their more or less spherical shape, unless under undue pressure or in contact with preformed canals or cracks in the cells.

In this connection the question arises as to why the Golgi apparatus exists for the most part in invertebrate animal cells as isolated bodies. Why does not the segregation apparatus (vacuome; Dawson, '28) in red blood cells of tailed amphibians fuse upon long exposure to osmic acid? These are obviously very difficult questions with our present knowledge of the vacuome. Equally difficult questions are suggested regarding the nature of the Golgi apparatus.

In an important study on the islet cells of the pancreas of the guinea-pig vitally stained with neutral red, Bensley ('11) observed that the canals of Holmgren (interpreted as the vacuome, '29) appear as colorless spaces among the deeply stained granules. As has been pointed out by Bowen, this observation fails to agree in the most obvious manner with the theory of Parat. If we assume with Bensley that the canals of Holmgren are the vacuome of Parat (which they probably are not; see Cowdry, '24), then we have a clear-cut case demonstrating that neutral red is not specific for the vacuome, as held by Parat and others. Since the vacuome in the living acinar cells of the pancreas exists as isolated vacuoles and granules, it becomes increasingly apparent that what Bensley was observing was perhaps quite a different thing from the vacuome of Parat and Painlévé and of Covell.[1] Also, as has been pointed out by Cowdry ('24), this observation of Bensley's ('11) will permit us to conclude that all clear canals are not fixation artifacts, since they have been observed by Bensley in the still living islet cells. This work of Bensley clearly convinces me that such a reticulum as that composing the Golgi apparatus is not impossible in the living cell, as held by Parat, and further, that the action of metallic methods does not cause artifacts of unknown nature in the cytoplasm of the cells. Furthermore, it has been shown by Strangeways and Canti ('27) that out of some fourteen cytological fixatives tested on tissue-culture cells, 2 per cent osmic acid produced the best fixation, caused the least distortion, and exhibited fewest artifacts.

In the acinar cells of the pancreas, Bensley has further described the 'internal reticular apparatus,' following fixation with formol-Zenker's fluid and staining with basic stains, as a network of closed spaces occupying the general zone of the classical Golgi apparatus. He compares this apparatus to that of Golgi, Kopsch, and Holmgren. Cowdry ('24), more recently, on the basis of studies of the same type of tissue as used by Bensley (the acinar cells of the pancreas), has suggested that the clear spaces and the Golgi apparatus are the negative and positive images of the same apparatus. If one assumes this view, however (Penfield, '21), then one sees (in some cases at least) the negative of the Golgi apparatus as a network following one of the best of our cytological fixatives and without the presumed drastic action of osmic acid and silver nitrate upon the surfaces of the vacuoles. Another alternative might be that the Golgi apparatus remains unstained by the methods used by Bensley and consequently appears as a clear reticulum in the colored cytoplasm. A similar condition is known to be the case in regard to the mitochondria. According to Bensley ('11)—

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[1] O'Leary has recently found in the living islet cells of the mouse (blood supply intact) that the canals of Holmgren "do not segregate neutral red applied vitally and also differ from the vacuome of Parat and others in that they usually appear as a closed network rather than discrete aligned vacuoles." Wistar Institute Bibliog. Advance Abst. Sheet, January 15, 1930.

> When the latter [mitochondria] are dissolved they leave irregular spaces in the continuous substance. When they are preserved but remain unstained, the spaces they occupy appear as clear rod-shaped spaces in the continuous substance and so give a deceptive appearance of longitudinal striations.

It is not unreasonable, therefore, to suppose that the conditions that apply so clearly to the mitochondria might also be applied to the Golgi apparatus. In fact, Jordan ('21) has suggested a somewhat similar hypothesis regarding this particular point.

Recently, Covell and Scott ('28) have made an experimental study on the relation of the vacuome to the Golgi apparatus. A smear of the spinal-ganglion cells of the ventral-horn substance of the mouse, that was intensively stained with neutral red, was made on a cover-glass and then inverted over a slide with a well containing osmic acid. The cells were directly exposed to the osmic-acid fumes. Continuous observations over extended periods of time permitted them to conclude that osmic-acid fumes preserve bodies vitally stained with neutral red and blacken them just as they do the Golgi apparatus. After seventy-five hours, they noticed an increase in size and in number of vacuoles and a tendency toward alignment. However, these bodies were never observed to fuse. Similar experiments were performed, using silver nitrate, which was found to preserve the neutral-red bodies and to blacken them as did the osmic acid. It is interesting to note that in these experiments Covell and Scott did not notice any tendency toward alignment and fusion of these bodies, for the latter retained the same relative position and identity as in the living tissues.

In Plasmodium precox, however—a parasitic protozoan in red blood cells of birds—Cowdry and Scott ('29) observed that the neutral-red bodies (vacuome), after treatment with osmic acid, aligned and fused to form a typical apparatus of Golgi. This seems a classical case in which the vacuoles were actually seen to fuse and form a reticulum. While these observations remain as convincing evidence in favor of the vacuome theory, I am somewhat skeptical as to whether the substance that was formed after the union of the vacuoles really represents the classical Golgi apparatus. From the studies of King ('26–'27), Park ('29), and others it seems probable that in the most favorable groups of Protozoa the Golgi substance exists as discrete bodies following metallic-impregnation methods. If we assume that the vacuoles actually fuse in the cells to produce a reticulum, it appears to me that this alone is not a sufficient criterion with which to identify the Golgi apparatus.

In some cases the massive appearance of what seems to be a reticulum may be observed in the acinar cells of the pancreas, following long exposure to osmic acid alone or to vital staining by neutral red and subsequent impregnation by osmic acid. Any one that is familiar with osmic-acid impregnation methods would immediately identify this as an artifact. Whether or not there is a true Golgi apparatus embedded in the mass of reduced osmium it is impossible at present to say. It is very evident that one cannot deny practically any type of a figure that might be represented following neutral-red osmic-acid technique, but we do object to the interpretation that it all represents Golgi material. Also, as has been demonstrated in this paper, the simple fact that bodies stained vitally by neutral red may subsequently be impregnated by osmic acid does not mean that they represent the classical Golgi apparatus. Here, unfortunately, our lack of precise criteria for the identification of the Golgi apparatus shifts the responsibility to a personal interpretation, which immediately explains the present status of our knowledge of the Golgi apparatus.

Dawson ('28) has recently urged the view that the so-called segregation apparatus in the erythrocytes of urodeles is homologous to the Golgi apparatus. His primary evidence for such an assumption rests on the fact that the segregation apparatus is readily stained with neutral red and blackened by prolonged exposure to osmic acid and silver nitrate. Dawson has noticed that various factors, including concentration of the dye, age of preparation, and the temperature at which the preparations are kept, influence the size of the segregation apparatus. Although the segregation apparatus is composed of clusters of granules, Dawson has never observed that they fuse, as was the case in Plasmodium precox. Since the segregation apparatus can be demonstrated by methods unfavorable for the demonstration of the Golgi apparatus in other tissues, it appears possible that the segregation apparatus may be a different structure from that of the Golgi apparatus. This assumption seems to be borne out in the researches of Sinigaglia ('10), where he finds that the Golgi apparatus in amphibian erythrocytes is composed of a network distributed throughout the cell. Also, Avel ('24) reports a discontinuous network in the erythrocytes of Rana fusca which he thinks represents the Golgi material. Then, too, Jordan ('25) suggests that the segregation apparatus is connected with the metabolic functions of the cell, analogous perhaps in a slight degree to the contractile vacuole in the Protozoa. In the light of these conflicting ideas, it is obvious that further evidence must be presented before a definite conclusion can be reached concerning the Golgi apparatus in erythrocytes.

Further complications regarding the vacuome have been noted by Chlopin ('27, '28). In an important study of the action of vital dyes on vertebrate and invertebrate cells, he finds that following the injection of vital dyes considerable amounts of a new substance may accumulate in the cytoplasm of the cells. The characteristics of this new product seem to vary according to the stages of deposition. In the early stages they showed no signs of protein that could be coagulated by the fixative. Later, the cells developed various amounts of substances which were preserved by fixatives and showed elective staining with basic dyes. This new substance has been given the name 'Krinom.' It seems to be the product of a special, physiological, intracellular, secretory process differing somewhat from that normally found in cells. The 'Krinom' substance seems to occupy generally a position similar to the vacuome and Golgi apparatus, and its location is affected by the physiological activity of the cell. Chlopin is of the opinion that there is no direct relationship between the 'Krinomsubstanz' and the Golgi apparatus. Accordingly, the results of Chlopin's study suggest that there are no such structures as the 'Krinomsubstanz' preformed in the cell, but that it is induced by the action of the vital dyes. These observations seem to find support in the recent work of Kunio Sato ('30). He finds that vital staining is due to a physical process rather than to a chemical one, and that the diffusibility of stains and the density of the cell play the most important rôles. He claims that stained granules found in the cell body are nothing but aggregations of the dye particles. These observations of Chlopin and Kunio Sato are important and merit careful consideration, as they offer a possible explanation for the appearance of the neutral-red bodies.

For a long time the vacuome theory seemed to find strong support in the fact that the vacuome appeared as the Golgi apparatus in plant cells (Dangeard, Guilliermond). More recently, this view has been decidedly weakened by the introduction of the osmiophilic platelets into the field of plant cytology following the brilliant researches of the late Dr. Robert Hall Bowen. It is becoming increasingly evident (Patten, Scott, and Gatenby, '28) that these osmiophilic platelets are the true Golgi apparatus in plant cells. At least they seem to meet the requirements of what should be expected in plant cells more completely than any other structures yet suggested. With the appearance of these new structures in plant cytology (osmiophilic platelets), it becomes increasingly probable that in plants as well as in animals the Golgi apparatus and vacuome are distinct and separate structures.

Very recently, Bhattacharya and Das ('29) have definitely demonstrated in the same cell the vacuome and Golgi apparatus as distinctly separate structures. Weatherford ('29) also points out the contradiction between the interpretation of the Golgi apparatus as an artificial product of the deposition of silver or osmium in or on the surfaces of the vacuome, and the great hypertrophy of the apparatus in the mammary gland preceding the appearance of a very large vacuole of secretion; and Horning and Richardson ('29) stress the difficulty of explaining the action of the Golgi apparatus in mitosis on the basis of an artifact. Then, too, it is also worthy of note that Golgi pieces can be demonstrated among the secretory products in the lumen of the gland—all of which points to its material reality (Brambell, '25; Bowen, '26, and Beams, '27).

Since the recent researches of Krjukowa ('29) on the salivary glands of the Chironomus larvae show that the Golgi material exists as separate bodies, typical of insect tissue (observations which we have confirmed, unpublished), considerable doubt arises concerning Parat's classical demonstration and interpretation of the 'Golgi apparatus.' In the light of all these facts, the conclusion seems inescapable that the Golgi apparatus and the vacuome are separate structures in the metazoan animal cell. In regard to the finer morphology and function of these structures it appears now that more definite answers must await further investigations.

SUMMARY

1. The Golgi apparatus in the acinar cells of the pancreas of the rat appears as a typical network with considerable variation in size and shape, located in the general area between the nucleus and the lumen of the gland.

2. Neutral red injected into the living animal does not stain the Golgi apparatus in the acinar cells of the pancreas.

3. The neutral-red bodies (vacuome) in the acinar cells of the pancreas of the rat can be stained intravitam by a subcutaneous or intraperitoneal injection of a saturated aqueous solution of neutral red or a saturated solution of neutral red in normal saline.

4. The vacuome in the acinar cells of the pancreas is not impregnated by osmic acid unless the tissue be previously stained intravitam by neutral red.

5. The vacuome is composed of red-colored granules following intravitam staining by neutral red. The neutral-red granules range in volume from bodies slightly larger to those considerably smaller than the mature zymogen granules. The vacuome may be localized at the apical pole of the nucleus on the boundary between the basal zone of the cell and the zone of zymogen granules, or scattered among the zymogen granules in the direction of the lumen.

6. The young zymogen granules have not been observed to bear any genetic relation to the neutral-red granules.

7. The total volume of the vacuome present in the cells is apparently affected by the different physiological conditions of the cell and the amount, concentration, and action of the injected neutral red.

8. Neutral-red preparations, showing neutral-red granules, followed by modified Mann-Kopsch technique, retain the identity of the neutral-red granules, colored a deep black. The neutral-red osmiophilic bodies (vacuome) in successful preparations appear morphologically and topographically the same as in the vitally stained neutral-red preparations.

9. Intravitam-stained neutral-red preparations of the acinar cells of the pancreas, followed by osmic-acid impregnation, disclose the vacuome and the Golgi apparatus side by side in the same cell. The vacuome may be embedded among the strands of the Golgi apparatus, but never fused with them.

10. It is concluded that the Golgi apparatus and the vacuome are two discrete substances in the acinar cells of the pancreas, both of which may occupy the same general position in the cell. Also, the classical Golgi apparatus in the acinar cells of the pancreas is not revealed by the action of osmic acid within and upon the surfaces of the neutral-red granules and vacuoles.

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PLATE 1

EXPLANATION OF FIGURES

All figures were drawn with a Zeiss 2-mm. objective, a no. 4 ocular, and with the aid of a camera lucida at table level. The drawings were made by Mrs. Alice Clarke Mullen.

1 Drawing of acinar cell of the pancreas of rat, showing a typical reticulated Golgi apparatus. This position of the Golgi apparatus in the acini cells seems to be the usual one in cells not overly active.

2 Acinar cell of the pancreas, with the Golgi apparatus stretched out into long strands which are usually connected and occupy the greater portion of the cell containing the zymogen granules.

3 Acinar cell showing a small and lightly stained vacuome at the apical pole of the nucleus, following intravitam staining with neutral red.

4 A common appearance of the vacuome following intravitam staining by neutral red. Its location is at the apical pole of the nucleus between the basal zone of the cell and zone of zymogen granules.

5 A more voluminous vacuome after the injection of neutral red. Some of the neutral-red granules are in their usual position in the vacuolar zone, others are scattered among the zymogen granules.

6 The neutral-red granules, following intravitam staining by neutral red, appear aligned as if about to fuse. They are, however, distinctly separate and no actual fusion has been observed.

7 A cell lightly stained intravitam by neutral red, in which the neutral-red granules are aggregated in groups surrounded by lightly red-tinted vacuoles.

8 Cell showing bodies of the vacuome stained intravitam by neutral red and subsequently fixed and impregnated one day in 2 per cent osmic acid. The position and morphology of the bodies are typically like that in the vitally stained neutral-red preparations. The Golgi apparatus has not been impregnated.

9 A cell which was lightly stained intravitam by neutral red and subsequently impregnated with osmic acid for two days. A small vacuome is present, and a typical Golgi apparatus is seen in its early stages of impregnation as a delicate thread uniformly impregnated.

10 In this cell, following neutral-red osmic-acid technique, the Golgi apparatus and neutral-red osmiophilic bodies occupy the same general position in the cell. The strands of the Golgi apparatus are distinctly seen among the bodies of the vacuome and the secretion granules.

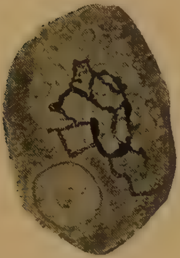
11 and 12 Similar cells showing the neutral-red osmiophilic bodies embedded distinctly among the strands of the Golgi apparatus.

13 Here the Golgi apparatus and the vacuome are seen extending well through the region of the zymogen granules in the direction of the lumen. The neutral-red osmiophilic bodies are also observed dispersed among the zymogen granules between, and parallel to, the strands of the Golgi apparatus.

14 This cell illustrates the vacuome and the Golgi apparatus occupying two distinctly separate positions within the cytoplasm of the cell. The vacuome is juxtanuclear in position and the Golgi apparatus is in its usual Golgi zone.

15 Cell in which the neutral-red bodies have been deeply stained followed by a long exposure to osmic acid. A massive appearance of reduced osmium is apparent in the cell, simulating a network, which is interpreted as an artifact.

16 Acinar cell of the pancreas following treatment with Regaud's technique for mitochondria. The mitochondria are typically filamentous.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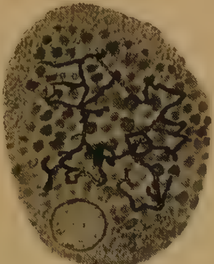
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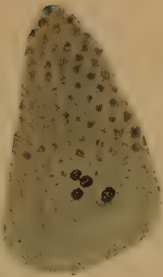
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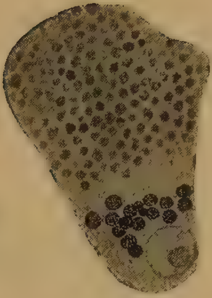
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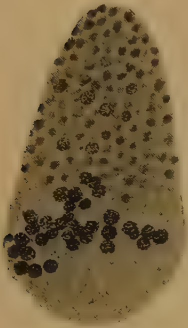
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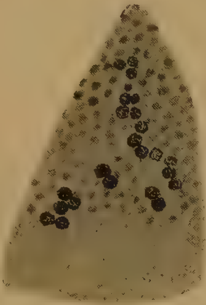
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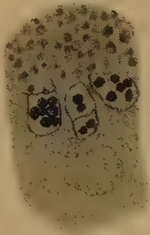
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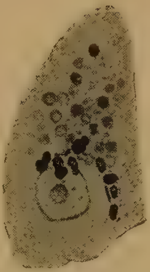
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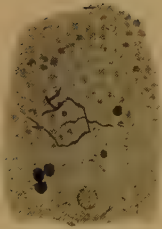
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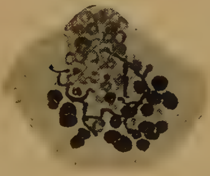
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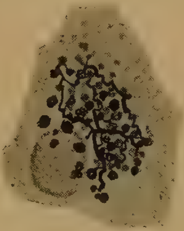
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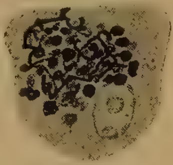
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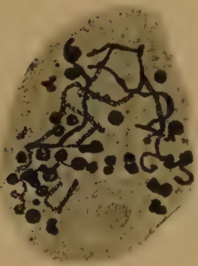
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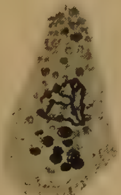
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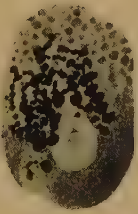
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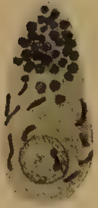
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STUDIES ON THE PHYSIOLOGY OF THE ACCESSORY GLANDS OF REPRODUCTION OF THE MALE GUINEA-PIG

JOHN WESLEY LAWLAH
Department of Anatomy, University of Wisconsin

TWO PLATES (SEVEN FIGURES)

This work is the outgrowth of anatomical researches on the accessory reproductive glands of some of our native squirrels (Sciuridae) (Mossman and Lawlah, not yet published). A study of the physiology of these glands in the gopher, chipmunk, or fox squirrel would have been undertaken had it not been for the obvious difficulties in working with wild animals. Hence the guinea-pig was selected as the experimental animal.

In spite of the large amount of work that has been done on the male guinea-pig, about the only proved functions of any of these glands are that the secretion of the seminal vesicles furnishes the bulk of the material which makes up the vaginal plug, and that the secretion of the cephalic pair of prostates ('coagulating gland' of Walker, '10) coagulates this vesicle secretion to form the definitive vaginal plug. This work has been confirmed by Engle ('26). Except in specific instances, no attempt will be made in this paper to review the literature, as this has been well done by several others: Walker ('10 and '11), Stockard and Papanicolaou ('19), Marshall ('22), Oslund ('23), Armistead ('25), and Engle ('26).

The present work consisted, then, in many cases, of a repetition of experiments which had previously been carried out by others. The main purpose of the author's work was to determine, by means of surgical removal and subsequent breeding and fertility tests, whether any of the accessory glands of reproduction are necessary for fertile coitus. Litter-size effects were not taken into consideration, since, using so few animals, such data would mean little or nothing. However, it is worth mentioning that there were no noticeable litter-size effects in any case. A secondary set of more or less preliminary observations were made upon these animals by means of anatomical and histological examination to see what effects the removal of one set of glands, or castration, or vasectomy would have upon the rest of the reproductive tract. Thirty-one male guinea-pigs were operated. Standard surgical procedure under ether anesthesia was used.

I wish here to express my sincere thanks to Dr. H. W. Mossman, of the Department of Anatomy, who assisted with the operations and helped in many other ways during the course of the work. Much of the success of the surgical work was due to the skill of Mr. William Kaplan and his excellently managed animal-surgery equipment.

I. REMOVAL AND LIGATION OF THE SEMINAL VESICLES

The seminal vesicles were removed from three large males, and in three other large males they were ligated at their proximal ends. The incision was made in the midventral line just anterior to the symphysis pubis. The glands were dissected away from their connections near the vas deferens, and here ligated with their vessels, and either removed or left in situ. In one case the symphysis was cut through and the glands ligated even more proximally where they enter the connective tissue surrounding the urethra. The muscle and peritoneum were sewed as one layer by interrupted catgut sutures. A continuous silk suture was used for the skin. The wound was dressed with collodion.

Upon recovery the animals were placed with females. The observations were essentially the same as those of Armistead ('25); the males in many cases made several unsuccessful attempts to copulate, then went to the corner of the pen, as if entirely oblivious to the proximity of the female. In other cases when apparently normal copulations took place no vaginal plug could be found. In no case were litters produced, although many females were allowed to come to heat in the pen with these males. Steinach ('94), using rats, reported reduced fertility manifest by reduced litter size after seminal-vesicle removal.

Leuckart ('53) expressed the opinion that the vaginal plug of the guinea-pig is secreted by the seminal vesicles and coagulated by the secretion of the prostate. Lataste ('83) believed that it is formed by coagulation of the secretion of the seminal vesicles. Besides confirming the work of Armistead ('25), that removal of the seminal vesicles renders the male guinea-pig sterile, this work also confirms that of Leuckart and Lataste in showing that the source of the bulk of the vaginal-plug material is the seminal-vesicle secretion.

Ivanoff ('00) and others have reported successful artificial fecundation of rabbits, guinea-pigs, and dogs with sperm taken directly from the epididymis diluted with an alkaline physiological salt solution. The conclusions from such experiments have, of course, been that the secretions of the accessory glands are non-essential to reproduction, except in so far as they furnish an alkaline activating and carrying medium for the sperm. The cause of sterility of guinea-pigs from which the seminal vesicles have been removed is not clear. Armistead believed it was due to the absence of the vaginal plug. That this cannot be the sole reason is shown by the work of Engle ('26), who removed the coagulating cephalic pair of prostates, thus preventing vaginal-plug formation, yet not producing sterility. Removal of the entire prostate by the author also gave similar results. It is probable that the physiology of the vesicular secretion is more complex and more interesting than has been commonly supposed.

II. REMOVAL OF COWPER'S GLANDS

Barrington ('13) removed Cowper's glands from ten guinea-pigs and found that six were able to produce litters. No description of his operative technique was given.

In the author's experiments six robust males were used. The incision was made in the middorsal line above the caudal vertebrae. Retraction of the skin exposed the muscles extending from the sacrum and coccyx to the coxa and femur. Separation of the muscle fibers laterally, from the third or fourth from the last caudal vertebra, exposed the tips of Cowper's glands. Grasping the tip of the gland with forceps and exerting a slight upward tension, it was easy with a dull probe to dissect out the duct down to its entrance into the fascia surrounding the bulbar portion of the urethra. In three animals the ducts were ligated before removal of the glands, while in the other three the glands and as much of the ducts as possible were picked away without injuring the covering tissue of the urethral bulb.

One animal died of unknown cause five days after the operation. The other five recovered and produced litters; one animal, three litters; two animals, two litters; two animals, one litter. Autopsy revealed no macroscopic evidence of regeneration of the gland in any of the animals. It should be kept in mind, however, that the most proximal ends of the ducts were not removed, and that microscopic sections of the penis of normal guinea-pigs show that the whole duct, including even this most proximal portion, has the same glandular structure as the body of the gland itself. Whether the small amount of secretion probably exuding from these duct remnants could compensate for the loss of the bulk of the gland in the reproductive function is an open question. Probably it could not, but so far we do not know.

III. REMOVAL OF THE PROSTATE GLAND

Six animals were used. A midventral incision was made over the symphysis pubis, the muscles separated, and the penis retracted caudalward to expose the symphyseal ligament, which was then cut. The pubic bones were then separated about 7 to 10 mm. and the membranous or muscular urethra was lifted slightly out of this opening by means of a small glass hook on the end of a rod of small caliber. The urethra was supported in this position by passing the rod in a transverse direction between it and the symphysis. In this manner the prostatic urethra was kept sufficiently exposed to allow the six lobes of the prostate to be rather easily picked away by means of fine curved forceps. In most cases a whole lobe could be removed at a time; in other cases the lobes were picked away in bits. Considerable care was necessary to avoid injuring the proximal ends of the vas deferens and of the seminal vesicles, both of which are surrounded by the prostatic tubules and are very delicate. After inspection showed the urethra completely stripped of its prostatic tubules, the glass rod was removed, allowing it to return to the pelvis. Hemorrhage was not very great. The pubic bones were drawn together by a piece of slowly absorbable catgut passed through the obturator foramina. The wound was closed by sewing the delicate subcutaneous tissue carefully with very fine gut, then by a continuous silk suture for the skin. The skin incision in this operation was unusually prone to open up, but the suturing of the subcutaneous tissue seemed to largely overcome this tendency.

Two of the animals died, one four days after operation, another three weeks after. The other four all recovered and produced litters; one animal, three litters; two animals, two litters; and one animal, one litter. Autopsy revealed macroscopically complete removal of the prostatic tubules and no regeneration. The prostate is not essential for fertile coitus in the guinea-pig.

Walker ('10) believed that he had shown that the cephalic pair of lobes of the prostate of the guinea-pig produces a secretion which causes coagulation of the secretion of the seminal vesicles, thus producing the vaginal plug. Engle ('26) also showed that the vaginal plug was never formed by males from which the cephalic pair of prostates had been removed, although he obtained several fertile matings from these males. As was suggested in section I, this indicates that absence of the copulation plug is not the sole cause of the sterility of males from which the seminal vesicles have been removed. A vaginal plug was never observed by the author in females mated to prostatectomized males. Still, there is always a possibility of missing the plug unless the animal is examined very soon after copulation.

IV. EFFECTS UPON THE ACCESSORY GLANDS OF CASTRATION, AND OF NECROSIS OF THE TESTIS BY LIGATION OF THE SPERMATIC CORD

Four animals were used, two young but mature males and two old males. The spermatic cords of one young and one old animal were ligated and the testes left in the scrotal sacs to undergo necrosis. The other two animals were castrated. All operations were performed through a small slit in the ventral surface of each scrotal swelling.

At the end of ten weeks the animals were killed and autopsied. In each case atrophy of all the accessory glands was clearly seen by gross examination (figs. 5, 6, and 7). Histological sections were also made. Although the general picture was as one would expect, yet there were points upon which accurate judgment was impossible, due to the small number of cases. Similar and much more complete studies of this problem have been made by Steinach ('94), Lode ('95), and Battelli and Martin ('22).

V. EFFECTS OF LIGATION OF THE DUCTUS DEFERENS ON THE ACCESSORY GLANDS

Six animals were used, three young but mature males and three old ones. Ligation was performed through a small midventral slit in the abdominal wall just cephalic to the symphysis pubis.

Autopsy at the end of ten weeks showed no macroscopic evidence of atrophy or hypertrophy of any of the accessory glands (figs. 3 and 4). Spermatozoa were alive in each testis.

Steinach ('94) and Oslund ('23) have obtained similar results in much more complete studies of this problem.

VI. EFFECTS OF REMOVAL OF THE PROSTATE ON THE REMAINING ACCESSORY GLANDS AND THE TESTIS

Seven animals were used, four mature young males and three old males. Four of these animals were used in the breeding experiments upon prostatectomized males. The procedure of removal has been described in section III.

At the end of ten weeks the three animals used for this part of the work alone were autopsied. The other four were killed after a longer interval, as they were being used in breeding experiments. Macroscopically, there was no evidence of change in the testis or the remaining accessory glands.

Serrallach and Parès ('07) and Hada and Goetzl ('14) claimed that prostatectomy produced testicular atrophy and other changes indicating an hormonal secretion of the prostate. Walker ('11), Lichtenstern ('16), and apparently all others who have worked upon this problem have denied such an effect. Some of the effects described by Serrallach and Parès are strikingly improbable. Hada and Goetzl's paper was unavailable.

CONCLUSIONS

1. Ligation or removal of both seminal vesicles in the male guinea-pig may somewhat modify their sexual behavior, but they are still capable of the act of copulation.

2. Ligation or removal of both seminal vesicles in male guinea-pigs in each case took away their capacity for forming a vaginal plug, and rendered them incapable of fertilizing the female.

3. Neither the removal of the prostate nor of Cowper's glands has any effect on capacity for copulation or for fertilization in the male guinea-pig.

4. Removal of the prostate had no macroscopic effect upon the testis or the remaining accessory glands of reproduction.

5. Both castration and spermatic-cord ligation produced definite gross degeneration of the accessory glands of reproduction.

6. Vas ligation produced no macroscopically visible change on the testis or the accessory glands of reproduction.

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PLATE 1

EXPLANATION OF FIGURES

1 Genital apparatus of normal male guinea-pig. Ventral view (seven-eighths natural size). *b.*, bulb of penis; *bl.*, bladder; *b-u.*, bulbo-urethral or Cowper's glands; *e.*, tail of epididymis; *h.e.*, head of epididymis; *h.s.*, horny stiles; *m.v.*, muscular or membranous urethra; *p.*, penis; *pr.*, prostate; *s.v.*, seminal vesicle; *t.*, testis; *t.v.*, tunics of testis turned back; *u.*, ureter; *u.o.*, urethral orifice; *v.d.*, vas deferens; *v.e.*, vasa efferentia.

2 Genital apparatus of normal male guinea-pig. Dorsal view (seven-eighths natural size). *c.pr.*, cephalic lobe of prostate ('coagulating gland' of Walker, 'proximal' lobe of Engle); *m.pr.*, middle lobe of prostate; *cd.pr.*, caudal lobe of prostate.

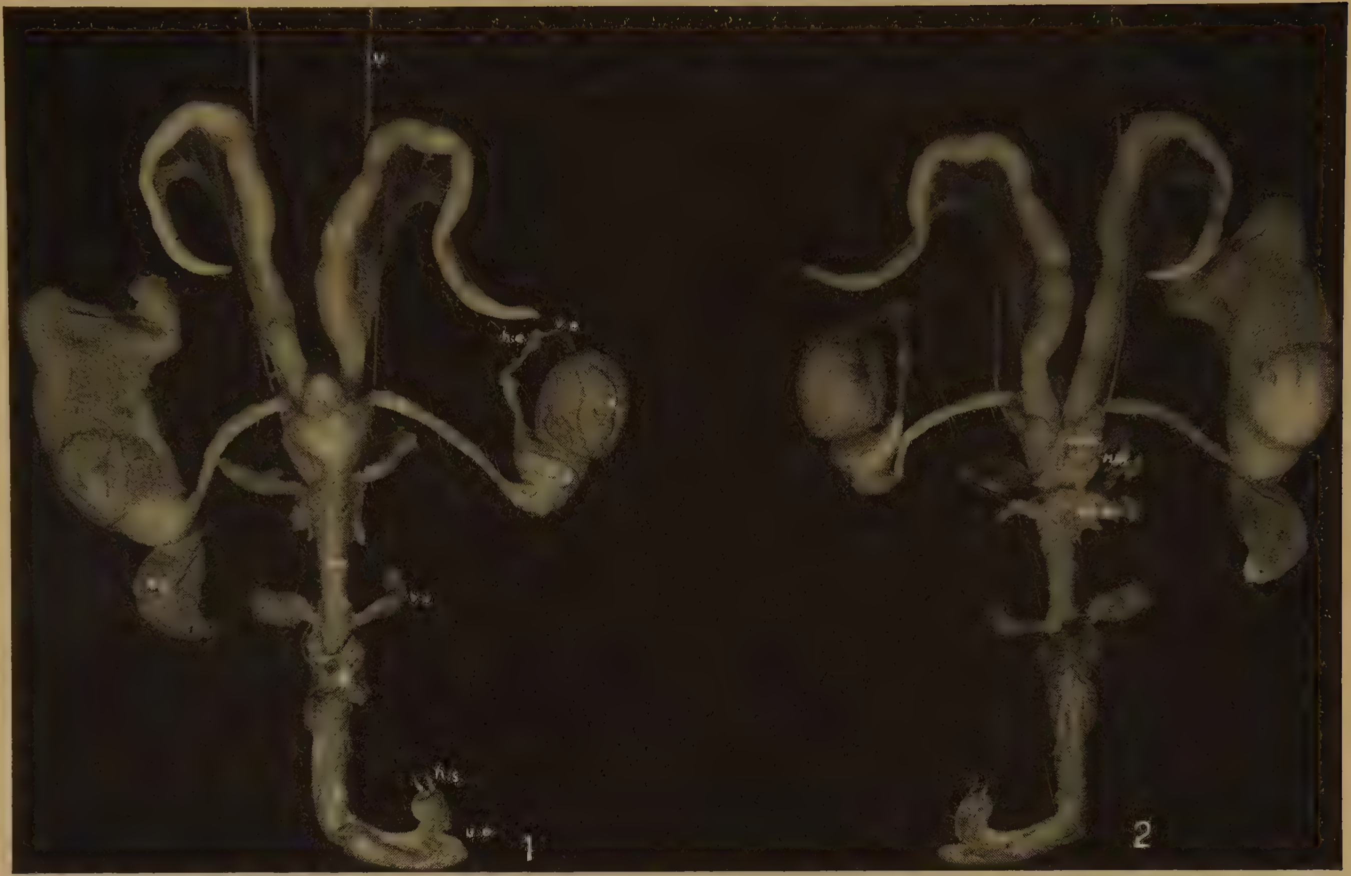
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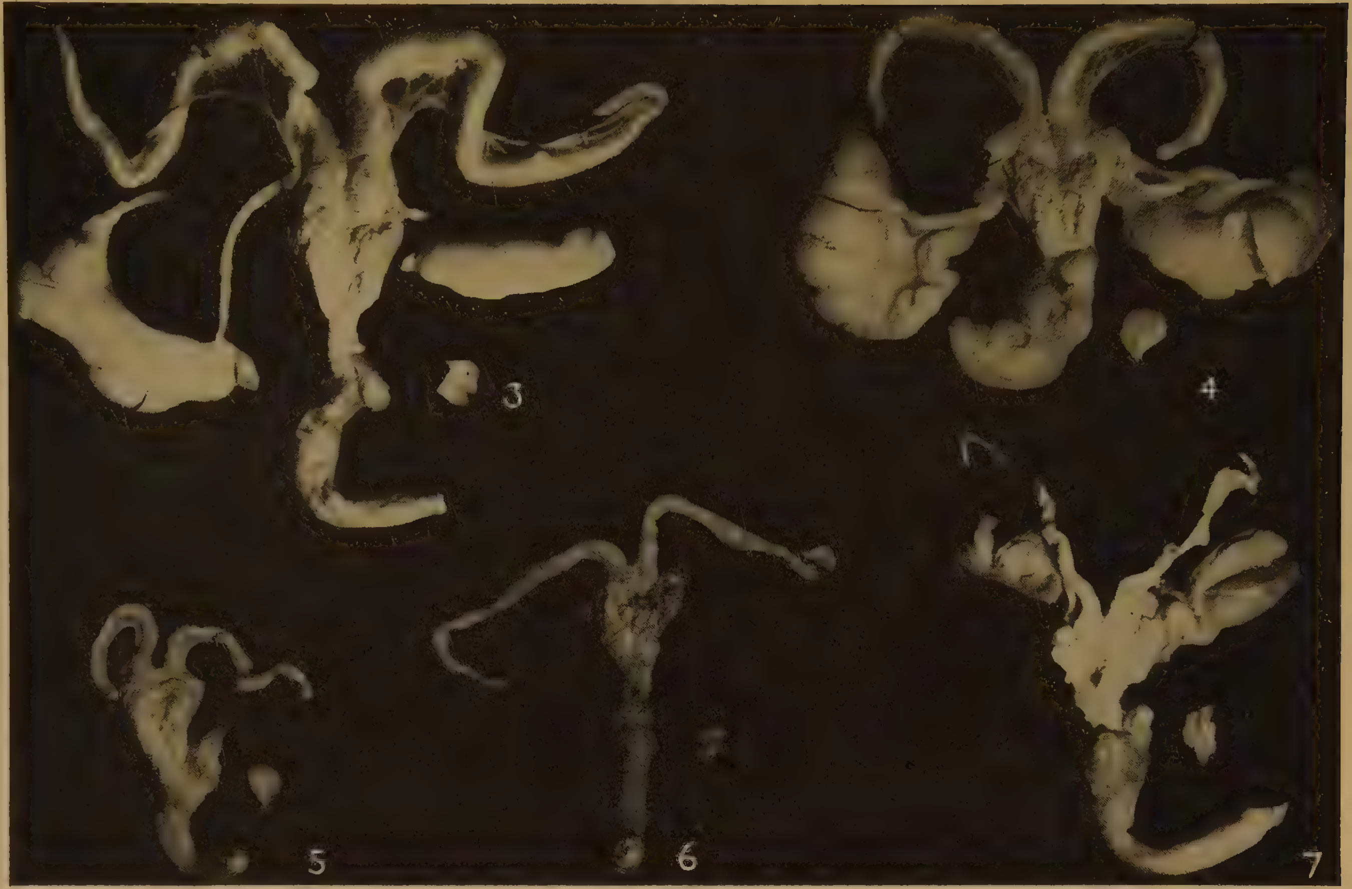
PLATE 2

EXPLANATION OF FIGURES

3 to 7 Genital apparatus of experimental guinea-pigs. In these dissections the fascias were not carefully dissected away as in figures 1 and 2. One Cowper's gland has been removed in each case and has been placed at the right of the bulb of the penis. All are dorsal views (all seven-eighths natural size).

3 Vas ligation. Young animal.
4 Vas ligation. Old animal.
5 Castration. Young animal.
6 Castration. Old animal.
7 Spermatic-cord ligation. Young animal.

PLATE 2



DIFFERENTIATION AND MULTIPLICATION BY MITOSIS OF CELLS OF THE ERYTHROCYTIC SERIES IN THE CIRCULATING BLOOD OF SEVERAL NORMAL URODELES

ALDEN B. DAWSON
Zoölogical Laboratory, Harvard University

TWO PLATES (NINE FIGURES)

In adult amphibians the spleen generally is the chief and at times the only locus of red-cell production, and under normal conditions the proliferation and maturation of these blood elements are confined more or less closely to the erythropoietic centers. However, in many larval forms transition stages are common in the peripheral blood and they may also show frequent mitosis. The present study was confined to the circulating blood of adult or almost fully grown animals, and out of the fourteen species of urodeles examined, ten showed mitosis in cells of the erythrocytic series and thirteen showed a morphological series of cell forms transitional between the hemoblast and the typical erythrocyte.

Atypical erythrocytes, characterized by varying degrees of basophilia, were reported by Nigrelli ('29) in the blood of Triturus viridescens. Many of these cells were undoubtedly degenerative forms resulting from the protozoon infection, but in some instances it was difficult to determine with certainty whether a particular cell was in an early stage of degeneration or was an immature erythrocyte. In the present study the atypical basophilic forms are unquestionably young cells, since the various types encountered have been observed in process of mitosis in some one of the several animals studied.

The other feature of interest in the study is the mode of division of the red blood cells. In a previous paper (Charipper and Dawson, '28) evidence was submitted that amitosis normally occurred in the circulating blood of Necturus, but that it was apparently restricted to the older, mature erythrocytes. Other workers, however, found that amitosis as well as mitosis frequently occurred in differentiating red blood cells (Jordan and Speidel, '29; Komocki, '26; McGowan, '26; Slonimski, '29; Stolz, '28, and Tuma, '26).

The material on which the present study is based is the same as that used for the determination of the amount and distribution of neutral-red bodies in the erythrocytes of urodeles (Dawson and Charipper, '29). At the same time the supravital studies were made, smears of blood from the same animals were also prepared and stained by both Wright's and Giemsa's methods. I am indebted to Dr. G. K. Noble for placing this amphibian material at my disposal and to Dr. H. A. Charipper for assistance in the preparation of the blood films.

The animals had been held in the laboratory for some time under uniform conditions, and were regularly fed. These facts probably explain why the blood of practically all the animals exhibited the same degree of physiological regeneration of the erythrocytes.

The blood was obtained during the last two weeks of June, a time at which regenerative changes in the blood should probably normally occur, as Zepp ('23) has shown that in frogs both the amount of hemoglobin and the number of red cells in the blood are lowest at this time of the year (May and June). Jolly ('04), however, has shown that regenerative changes, accompanied by mitosis of the erythrocytes in the peripheral blood, can be induced in Triton by either severe bleeding or by refeeding after a prolonged fast.

Some reference should also be made regarding the region of the animal from which the blood was drawn, since differentiation of the immature elements of the blood is apparently favored by a sluggish circulation such as occurs in the venous sinusoids. In many of the smaller animals (Pseudobranchus striatus, Desmognathus fuscus, Desmognathus phoca, Desmognathus quadramaculata, Eurycea bislineata, and Plethodon glutinosus), the blood was taken directly from the heart; in Necturus and Amblystoma tigrinum (unmetamorphosed), from an afferent branchial artery; in Cryptobranchus, from a vein in the roof of the mouth; in Pleurodeles waltlii, from the abdominal vein; and in Amphiuma, Siren, and Triturus, through incisions in the skin.

It seems unnecessary to separately describe the findings for each animal, as they can be readily summarized. Typical examples are also included in the plates. With the exception of Pseudobranchus, intermediate stages in the maturation of the erythrocytes were found in the blood of all animals examined, but mitotic figures were never observed in smears from Amblystoma, Amphiuma, and Necturus. Transitional forms and mitoses were most numerous in the blood of Desmognathus quadramaculata, Plethodon glutinosus, and Pleurodeles waltlii, but were found more or less frequently in all the other animals with the exceptions previously noted.

The least differentiated of the erythrocytogenic cells appearing in the blood stream differed but slightly in their histological characters from the large and medium-sized lymphocytes. The nuclear pattern showed some slight advance toward that of the typical erythrocyte and the cytoplasm was characterized by its extreme basophilia and its turbidity. The intermediate stages were characterized by a nucleus in which the reticulum became progressively more distinct and coarser, but for a time the cytoplasm tended to remain strongly basophilic and the apparent turbidity was gradually replaced by a coarse granular appearance which at times became almost flaky.

In these earlier stages the outline of the cell is not smooth, but is irregular. However, as the cytoplasm gradually loses its basophilia, with the increasing elaboration of hemoglobin, the cell outline becomes smooth and the coarsely granular or flaky appearance of the cytoplasm becomes successively finely granular, homogeneous, and at last almost hyaline. The differentiation of the nucleus and cytoplasm is not always closely correlated, as one or the other may lag considerably in its maturation, giving rise to more or less atypical transition forms. Some of this irregularity is perhaps only apparent rather than real, since many of the atypical cells may represent either cells undergoing changes preparatory for mitosis or daughter cells reorganizing after a mitotic division.

So far nothing has been said of the changes in form and size that these cells also undergo. Ordinarily during maturation, the cell, which is at first spherical, changes to a spindle form (fig. 1) and finally becomes a flattened oval. At the same time the nucleus usually becomes small and dense, and eventually acquires the oval form to conform with the changed outline of the cell itself.

In the intermediate stages there does not appear to be any definite correlation between cell size and degree of maturity, although the fully differentiated erythrocytes are usually remarkably uniform in size. This lack of correlation is apparently dependent on two factors, rate of growth and degree of mitotic activity. In the younger stages the erythroblasts may apparently undergo successive divisions without any appreciable intervening growth period (figs. 5, 6), since very small cells but slightly larger than thrombocytes are frequently encountered. On the other hand, very large polychromatophilic cells, larger than the mature forms, are often found (fig. 9). These irregularities, however, are normally smoothed out, both by growth of the small elements and by division of the abnormally large cells. It does not seem necessary to explain these significant departures from normal size as the result of unequal amitotic divisions as McGowan ('26) has done for the blood of the fowl. Binucleated cells are occasionally encountered (fig. 8), but they may be due to the failure of the cytoplasm to divide following indirect nuclear division.

Striking variations in the nuclear-cytoplasm ratio are also characteristic of these maturing erythrocytes. These, like the other irregularities noted, are at least partially explained by the very evident mitotic activity which characterizes the blood of these urodeles under consideration. Cell growth and changes in the form of the cell and of the nucleus may also be factors producing the variations in karyoplasmic ratio. In cases where the maturation of the nucleus and cytoplasm is not closely correlated the change of the form of the cell to a flattened oval may produce an apparent but not actual increase in cytoplasmic volume, since the cells in a smear are ordinarily viewed en face.

DISCUSSION

From this survey of the conditions in the circulating blood of these several urodeles it is apparent that in what appears to be normal, physiological regeneration of the red blood cells both proliferation and maturation of the elements occur intravascularly to a considerable extent. Similar conditions are described by Jordan and Speidel ('29 b, '29 c) for both the lamprey and the hagfish; and Komocki ('26) finds evidence of extensive transformation of hemoblasts into erythrocytes in the blood of Emys orbicularis, but in this form proliferation is by the amitotic method. Jordan and Speidel ('29 d) also find that in splenectomized salamanders the thrombocytes differentiate from lymphocytes and multiply by mitosis in the blood stream.

In many ways the erythrocytic picture presented closely resembles that found in cases of pernicious anemia of the warm-blooded vertebrates. It seems probable, therefore, that the relatively unspecialized erythropoietic centers of the lower vertebrates are less able than the normal bone marrow to retain the red cells until they are fully mature, and the polychromasia, poikilocytosis, and anisocytosis which mark the blood picture during rapid recovery from a relapse in pernicious anemia may be due in part to the 'myeloid metaplasia' which may occur in both the liver and the spleen, although the hyperplasia of the marrow doubtless also is an important factor.

No conclusive evidence on the ancestry of the erythrocyte appears in this study. A morphologically complete series of stages between the lymphocyte and the mature erythrocyte can be arranged and there is nothing that tends to controvert Jordan's conclusion that the lymphocyte is the hemoblast.

The only mode of division encountered in the maturing erythrocytes was the indirect method. The illustrations give abundant evidence on this point. In many mature cells, however, the picture presented is the same as that previously described by Charipper and Dawson ('28) and interpreted as evidence of direct division.

SUMMARY

The circulating blood of thirteen out of fourteen different species of urodeles examined during the month of June gave definite evidence of intense physiological regeneration of the erythrocytes. All stages, from the lymphoid hemoblast to the fully differentiated red blood cell, were found in the circulation. The only mode of cell multiplication in the maturing cells was the indirect one. Examples of this were numerous. The cell patterns presented by the red blood cells were very variable, basophilia, polychromasia, anisocytosis, and poikilocytosis being the most marked features. These irregularities are apparently due to the progressive differentiation of young cells, to variations in the amount of growth of individual cells of different ages, and to the degree of mitotic activity occurring in the cells of the several stages. The uniform blood pictures presented by representatives of seven different families is probably due in part to the fact that these animals had been held for some time in the laboratory under the same conditions.

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EXPLANATION OF PLATES

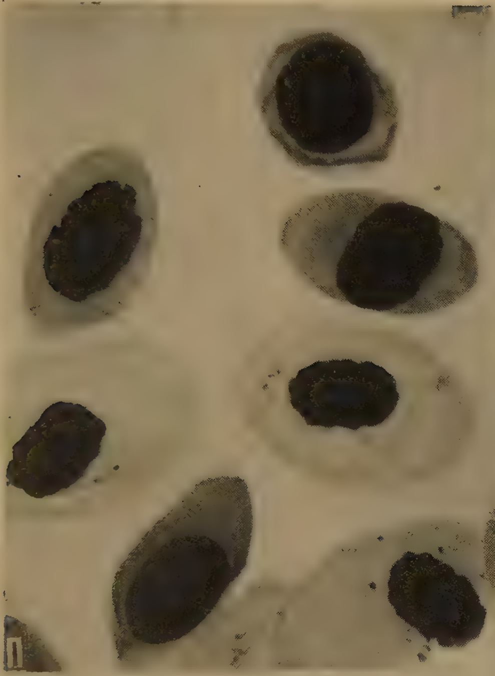
All figures were photographed at the same magnification. All preparations shown were stained with Wright's blood stain.

PLATE 1

EXPLANATION OF FIGURES

1 Erythrocytes of Pleurodeles, showing the variations in size, form, and staining reactions of these cells. Note the presence of discrete basophilic granules (segregation apparatus) in the clear cells. In the cells showing polychromasia only a fine, uniform granulation is evident.

2 to 4 Erythrocytes of Pleurodeles, showing many of the features described for figure 1. In addition, three mitotic figures are shown. The cytoplasm of the cells undergoing mitosis is finely granular and decidedly basophilic.



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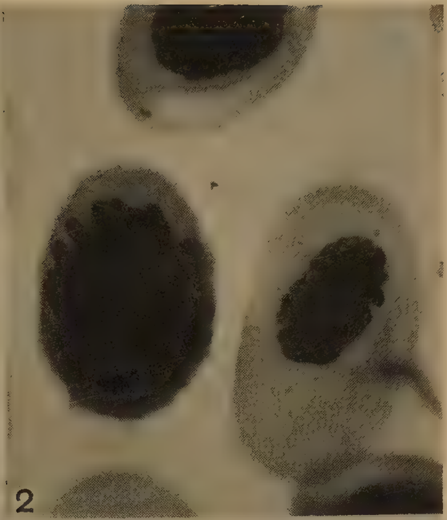

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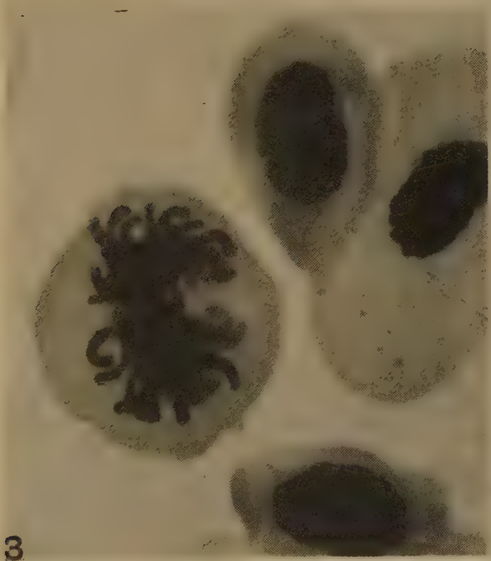

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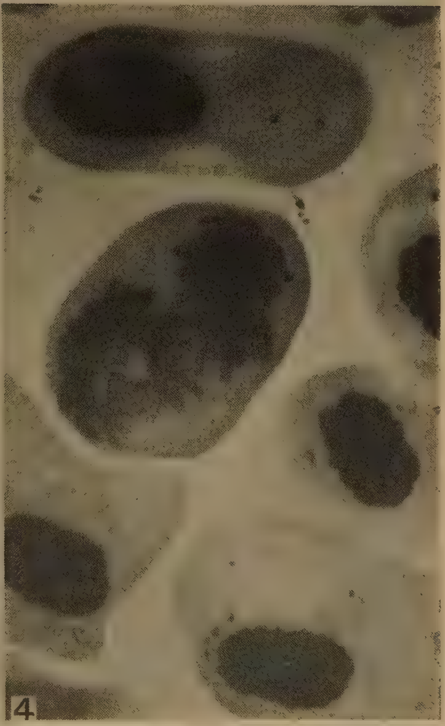

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PLATE 2

EXPLANATION OF FIGURES

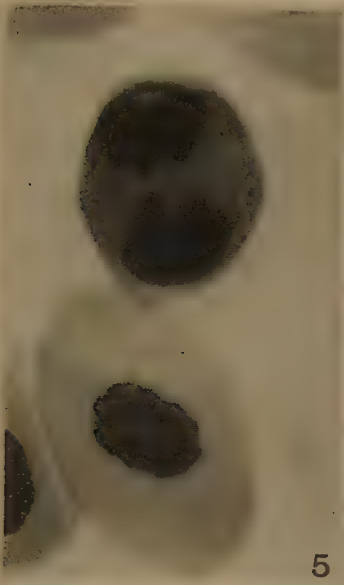
5 An immature red blood cell of Desmognathus quadramaculata undergoing mitosis. Note the relatively coarse granulation and the degree of basophilia. A mature erythrocyte is also shown.

6 A later stage of mitosis in an immature erythrocyte from the blood of Desmognathus quadramaculata.

7 A dividing erythrocyte from the blood of Plethodon glutinosus.

8 A binucleated polychromatophilic erythrocyte from the blood of Plethodon glutinosus, probably due to the failure of the cytoplasm to divide following indirect division of the nucleus.

9 Two immature erythrocytes from the blood of Cryptobranchus allegheniensis; one undergoing mitosis, the other apparently in an early stage of the prophase.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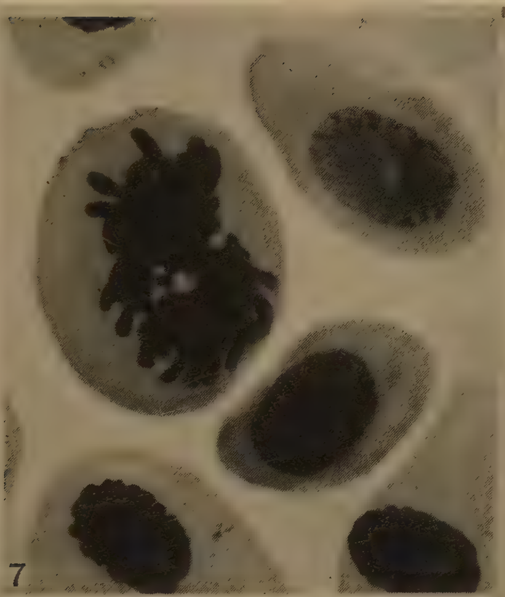
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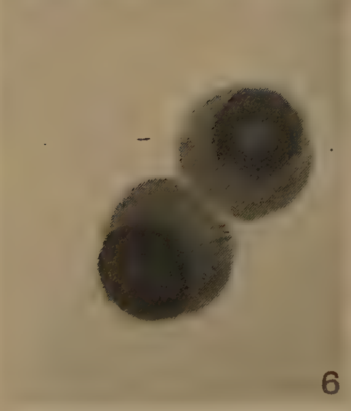
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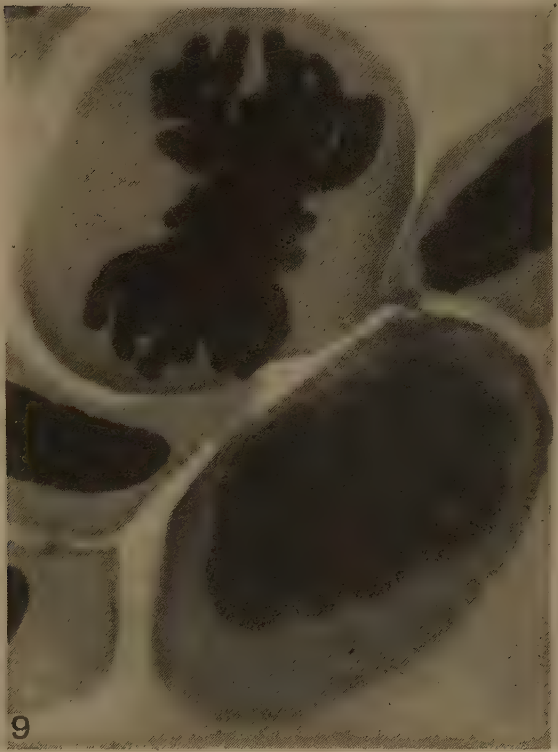
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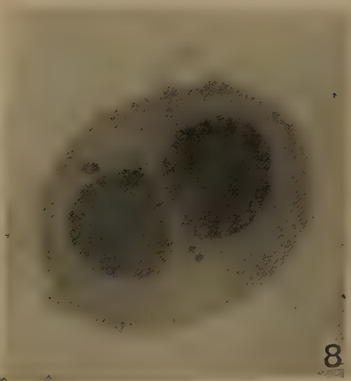
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THE DEVELOPMENT OF THE TONSIL OF THE DOMESTIC PIG

PAUL M. LEVIN
Carnegie Embryological Laboratory, Baltimore, Maryland, and Anatomical Laboratory, University of Rochester, Rochester, New York

TWO PLATES (SIX FIGURES)

Investigators who have heretofore given accounts of the development of the tonsil have invariably placed great emphasis upon its derivation from the pharyngeal-pouch system. They have traced the region occupied by the tonsil back in the embryonic series to the sinus tonsillaris, which was found to be a remnant of the second visceral pouch. Likewise, other organs, such as the parathyroid glands, thymus, etc., were described as derived from particular branchial pouches. Like them, the tonsil became included in the theory of branchiomerism, and our conception of its homology and physiology has been correspondingly influenced.[1] Among the various authors there has been considerable diversity in observation, and, more recently, the question has arisen, is not the development of the tonsil in tissue of branchial-pouch origin fortuitous and without any specific branchiomeric significance? Kingsbury and Rogers, in a recent paper ('27), have reached such a conclusion. They write, "A complete linkage of palatine tonsil and second pouch appears to be without justification in fact."

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[1] Briefly, the results obtained were as follows: His, in his classical work on human embryology ('80), considered the tonsillar sinus (the site of the tonsil) as the transformation of the second visceral pouch. To this view, Stöhr ('91) accorded.

Born ('83) and Kastschenko ('87) concluded from their studies that pouch II disappears without giving rise to the sinus tonsillaris.

Hammar ('03) traced the sinus in various mammals to a dorsal prolongation of the second pouch. Grosser, in Keibel and Mall's Manual ('12), accepted Hammar's results, as, indeed, have almost all others interested in tonsillar origin.

Grünwald ('10) concluded that the tonsillar anlage is derived exclusively from the ventral part of pouch II, and that this points to an originally specific structure and function.

Kingsbury and Rogers ('27), in studying the development of the bovine tonsil, found that the ventral part of pouch II and probably a part of pouch III form the site of the tonsil.

Toward the solution of this problem the tonsil of the pig, owing to its adult topography, presents certain advantages, and thus the pig is a favorable species in which to trace tonsillar development. In the pig the organ is situated in the tissue of the soft palate, as distinguished from its position in the lateral wall of the pharynx in most other forms. The palate being relatively flat (fig. 1), the adenoid tissue thus develops in the absence of what may be called a sinus tonsillaris. All direct topographical connection between the tonsil and the branchial-pouch system is thus lost. We may consider, in the pig, the tonsil as a self-sufficient organ.

Accounts of the development of the pig's tonsil are given by Retterer ('88) and by Hammar ('03). Their descriptions are in the main corroborated by my own material. Retterer has, however, colored his observations with the idea of the origin of the lymphocyte from epithelium. Thus he depicts transitions from the lining epithelium to submucosal lymphoid aggregations. This interpretation is no longer accepted by students of the lymphoid-tissue problem as representing the actual state of affairs.

Hammar describes, in addition to the tissues considered here as the palatine tonsils, a pair of small lymphoid structures in the lateral walls of the pharynx of the pig. These latter he considers the true homologues of the palatine tonsils of other mammalian species; the more conspicuous adenoid tissues he considers accessory. However, investigators of porcine anatomy (Illing, '03; Sisson, '17) regard the palatine structure as the true tonsil. I have not been able to find in the embryological literature any reference to this distinction.

The tonsil of the pig, is, then, an oval structure, characterized by numerous crypts opening independently on the surface (fig. 3). Its dimensions in the adult are approximately 4.5 × 2 × 0.7 cm. Study of the development of the tonsil in this species is desirable because of, 1) its development in the absence of a tonsillar sinus and, 2) its relative simplicity of structure.

MATERIAL

The material for this study consists of forty-five pig fetuses, obtained through the courtesy of the Rochester Packing Company, Rochester, New York. These specimens varied in size from 60 mm. to 275 mm. CR length. The method of preparation of these specimens for study was as follows: The region of the soft palate was exposed and plunged into fixative. Fixative solutions used were corrosive-acetic mixture, 10 per cent formalin, and Bouin's fluid. After the usual procedures for dehydration and paraffin embedding, blocks were cut in sections 7 to 10 μ thick. In thirty selected cases serial sections were prepared. Hematoxylin and eosin were used on all the specimens, and, in addition, a few were stained with Mallory-Wright connective-tissue stain. Two heads of adult pigs, apparently normal, were obtained from the abattoir of J. A. Gebelein, Baltimore. As with the fetuses, the palate was exposed and dissected, then fixed in 10 per cent formalin. Sections were cut from blocks embedded in celloidin. Newborn or young pigs not being available, the gap between the embryonic and adult stages could not be filled.

HISTOGENESIS OF THE TONSIL

Histological changes leading to the formation of tonsillar tissue first appear in fetuses of 90 mm. CR length. Before this, however, there is evidence of differentiation of the palatine tissue in the site of the tonsil. The mucous glands of the soft palate develop about, but not within, this region. They thus outline a bare and smooth oval portion of surface epithelium (fig. 2). Here the following changes occur, as revealed in the histological sections:

At about 90 mm. there begins a diffuse proliferation of the basal epithelial layer and a subepithelial condensation of mesenchyme. These processes soon, at 100 mm., are accentuated in numerous areas, forming small nodules which project slightly from the surface (fig. 4). The epithelial growths do not yet extend into the underlying tissue. There are a few lymphocytes and polymorphonuclear leucocytes about the blood vessels of the mesenchyme, in the meshes of the latter, and in the mesodermal nodules. These lymphocytes and granulocytes are constant findings, increasingly numerous, in the remainder of the embryonic series (see later, p. 193).

The clumps of basal epithelial cells begin to grow into the mesenchyme at 115 mm., forming knobs composed of rather densely staining cells with oval, vesicular nuclei. The mesodermal aggregations become increasingly more discrete and dense. They first form spherical masses at the tips of the epithelial proliferations. But as these extend farther into the underlying regions, the mesodermal nodules hollow and enclose the epithelial growths in cup fashion (145 mm.) (fig. 5). There is a moderate lymphocytic infiltration of the ends of the epithelial processes. The mesodermal nodules grow, 1) by mitotic division of their cells, 2) possibly by further new development of lymphoid cells, and, 3) possibly also by addition of cells from without, there being at all stages abundant perivascular leucocytic deposit.

The first signs of crypt formation are changes occurring at the centers of epithelial outgrowths at 142 mm. The cells at the center enlarge with the formation of pink-staining cytoplasm (keratinization?). This process of cell transformation extends to the surface (fig. 5). A dimple-like depression is formed at the center by the sloughing of cells, which progresses inward, leaving the proliferation as a hollow pit lined by pink-staining squamous cells and a layer of basal cells. The basal cells become uniformly oriented in the layer, and the lining of the crypt then closely resembles the surface epithelium (fig. 6).

The sprouts grow by the multiplication of basal cells and invasion into mesoderm. The basal layer is maintained at a thickness of one cell by the squamous transformation of the older cells. The squamous cells lining the crypt are continuously sloughed off, thereby extending the lumen.

Occasionally the direction of growth of the epithelial proliferations may change, leading to the formation of branches. When this occurs, the lymphoid nodules surrounding the growing tips likewise divide and enclose the branches (fig. 6). The new offshoots are, of course, infiltrated with lymphocytes.

The formation of new epitheliolymphoid units (epithelial crypts and mesodermal nodules) occurs in fetuses as large as 185 mm., but not in older specimens.

Definite study of the vascularization of the tonsil has not been made. However, the region of the tonsil is well supplied with blood vessels, especially after the stage of crypt formation. The capillaries are numerous from the level of the underlying muscles to the surface. They penetrate the periphery of the mesodermal nodules, but rarely the center.

As the epithelial growths elongate, the lymphoid nodules are separated from the surface epithelium, with the development of a fibrous-tissue layer between. There is some fibroblastic proliferation beneath the tonsillar tissue, with the formation of the capsule. From this trabecular connections to the superficial layer grow between small groups of crypts.

CELL TYPES

Three types of cell are outstanding in the embryonic series: 1) mesenchymal cell, 2) lymphocyte, and, 3) polymorphonuclear leucocyte (both neutrophil and eosinophil types). The mesenchymal cells are derived from the palatine mesenchyme. They undergo proliferation during the period of tonsillar formation, especially in the early stages, apparently simultaneously with the epithelial growth. The lymphocyte first appears at about 100 mm. Both large and small forms are present. They are found distributed in the meshes of the mesenchyme, but some are collected about blood vessels. The interpretation of this perivascular distribution is briefly discussed later. Mitoses soon occur in the lymphocytes. As a result of this, nests of cells, closely packed, are formed in the mesodermal reticulum and in the subepithelial regions. Lymphocytes gradually become the predominant cell type in the aggregations, which, by 275 mm., are definitely 'lymphoid nodules.' In the tonsil of the mature fetus the lymphoid follicles thus consist chiefly of compact lymphocytes, with no germinal centers. This corresponds to the structure of follicles, at birth, in lymph nodes of the pig. These, according to Baum and Hille ('08), do not yet possess germinal centers.

Leucocytes of the granulocytic series first appear in tonsils of embryos about 135 mm. in length in the perivascular regions. They are fairly numerous at about 160 mm. Some are distributed in the reticular tissue and mesodermal nodules, but they are at no time as abundant as the lymphocytes. Mononuclear eosinophil cells are present and relatively numerous; they do not differ in distribution from the polymorphonuclear ones. One cannot, of course, definitely disprove the local origin of some granulocytes. From their distribution it would seem that they have migrated in from the blood stream. Basophilic cells are not found, possibly because of the characteristic granules having been dissolved out in the preparation of specimens.

In fetuses of about 160 mm. CR length, several extravascular collections of erythrocytes were found, the cells being distributed through the internodular reticulum. There are also occasional hemoglobin-containing, nucleated cells—'erythroblasts.' That this is not an extravasation of blood occurring with death is proved by the occurrence in these regions of large cells containing yellow pigment particles similar to disintegrated hemoglobin. There are at least two possible explanations of this phenomenon. First, there may have been an extravasation or diapedesis of blood during life. As erythroblasts occur in the blood stream at this stage of development (Engel, '99), their presence does not disprove this possibility. Secondly, there may be local erythropoiesis. However, this latter assumption is not a necessary one.

The tonsil of the adult pig is, as mentioned above, a relatively flat structure. It is covered with stratified squamous epithelium, with numerous deep papillae at its base, and is surrounded by a fibrous capsule. Through the lymphoid tissue run many fibrous trabeculae. The epithelium is invaginated into deep crypts, some of which branch once or twice. The reticular tissue is compactly filled with lymphocytes. There are numerous so-called germinal centers, arranged about the crypts. In these centers the cells are large, pale, with irregular nuclei. Mitoses are fairly frequent in these pale areas, but rare in the dense lymphocytic aggregation.

Lymphocytes may be seen permeating the epithelium, especially of the crypts. Polymorphonuclear eosinophilic granulocytes are quite numerous. They seem chiefly beneath, and invade, the epithelium of the crypts; but they are also seen in the germinal centers and dense lymphoid tissue. Neutrophilic granulocytes are less common, being found chiefly in the contents of crypts.

DISCUSSION

The pattern of mucous-gland distribution prior to the definitive changes in the development of the tonsil, i.e., in fetuses of 60 to 80 mm. CR length, is of particular theoretical interest as to the specificity of tissues. The arrangement is such that the site of the tonsil is left free of glands (fig. 2). This observation was recorded by Retterer in his paper on tonsillar development ('88, p. 310). It is thus evident that the developmental potencies of the tonsillar primordium are determined before histological change appears. Possibly there is an influence inhibitory to mucous-gland development.

Mention may be made of the curious perivascular aggregation of lymphocytes at certain stages in the development of the tonsil, as noted above (p. 193). Latta, in studying the histogenesis of lymphoid tissue in the intestine of the rabbit ('21), noted the occurrence of the same phenomenon in this material. Two explanations of this aggregation have been offered. First, the cells may be leaving the blood stream, localizing in the lymphoid primordium; secondly, the cells may have developed in the region and are secondarily attracted by an unknown influence to the perivascular area. Latta, perhaps rightly, favors the latter possibility. His reason is that the lymphocyte first appears in very little or no definite relation to the blood vessels, only later to aggregate about them. As noted above, this appears to be the state of affairs also in the palatine tonsil of the pig. Uta ('29) describes in the human tonsil the first appearance of lymphocytes in the perivascular regions, assuming thus their hematogenous origin.

In summary, the story of the development of the pig's tonsil is as follows: The branchial topography appears and disappears, and the palate forms. After a period of inactivity, certain epithelial and mesenchymal changes concur in both sides of the midline of the soft palate. These changes, with possibly the addition of certain cells from the blood stream, lead to the formation of epithelium-lined crypts surrounded by lymphoid nodules.

The tonsil thus develops in no direct relation to the pharyngeal-pouch system. While it may be said, doubtless truly, that the tonsil arises from tissues which formerly composed part of the pharyngeal pouches, yet this in itself does not indicate the existence of a causal relationship between the two.[2]

The surface of the soft palate prior to tonsillar formation, as noted, is relatively flat. This demonstrates the independence, in the pig, of the palatine tonsil from the topographical entity of other mammals—the tonsillar sinus.

CONCLUSIONS

In light of these facts, the palatine tonsil in its essence should be considered a morphologic entity. It is not merely a derivative of the pharyngeal configuration known to tradition as the 'branchial-pouch system.' Nor should the tonsil be regarded as a development of the sinus tonsillaris.

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[2] Rogers ('29) has quite recently discussed the pharyngeal transformations in the rat—a species which has no palatine tonsils. He found that the second branchial pouch disappears fairly early in development, and that no tonsillar fossa is formed.

To Drs. G. L. Streeter and G. W. Corner the author wishes to express his earnest appreciation. This problem was undertaken under their direction, and throughout I have been aided by their suggestions and advice.

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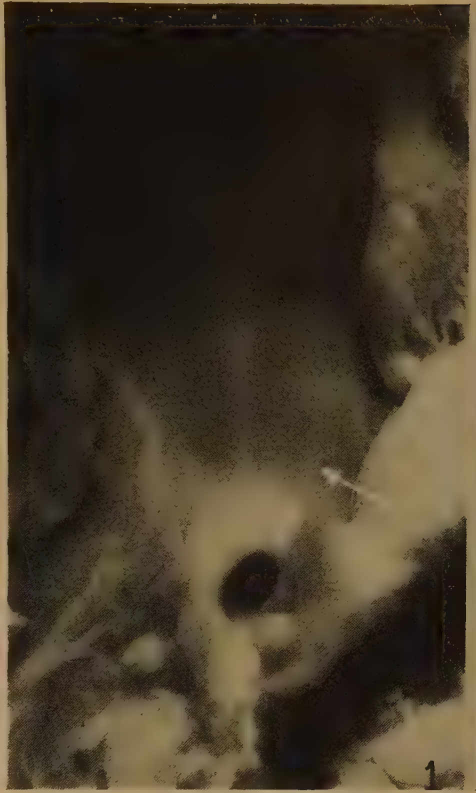
PLATE 1

EXPLANATION OF FIGURES

1 Palate, 65-mm. fetus. Formalin. × 8. Shows flat nature of palatine surface; absence of sinus tonsillaris. Border of site of tonsil marked by row of mucous glands in a very early stage of development.

2 Palate, 85-mm. fetus. Formalin. × 5. Further development of mucous glands, whose papillae show brightly in photograph. Palate otherwise flat.

3 Palate, 200-mm. fetus. Mercuric chloride. Stained with aqueous alum-cochineal. × 4. Shows tonsillar areas with well-formed crypts. These areas are free of mucous glands, which are present about the tonsils.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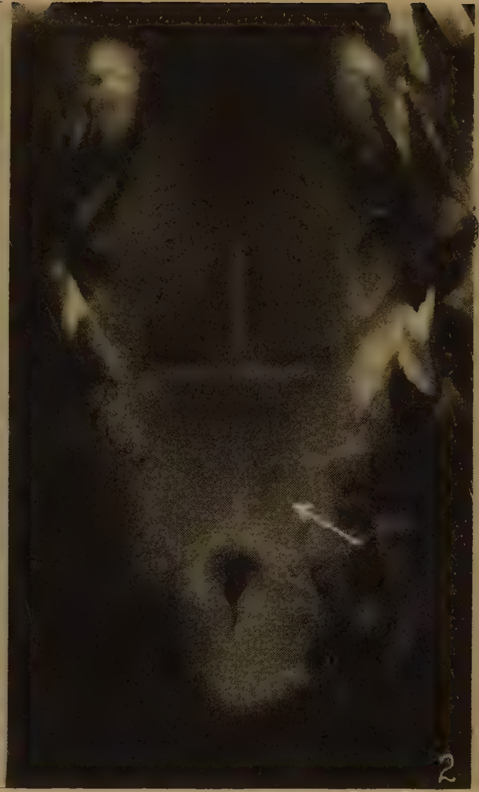
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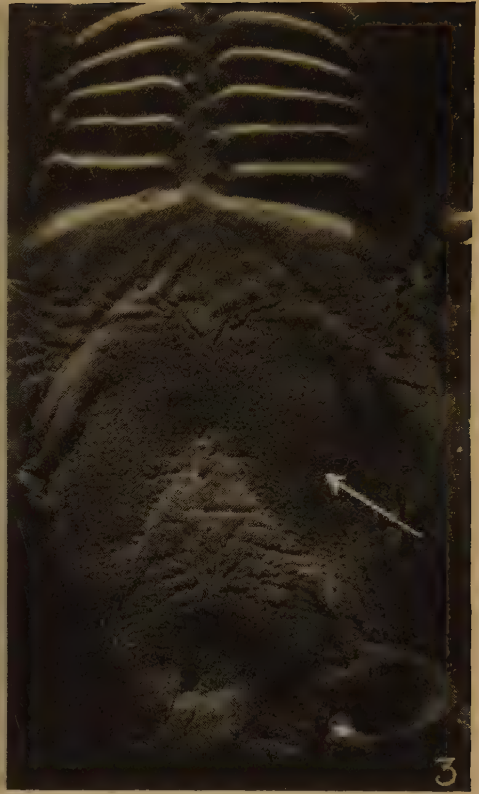
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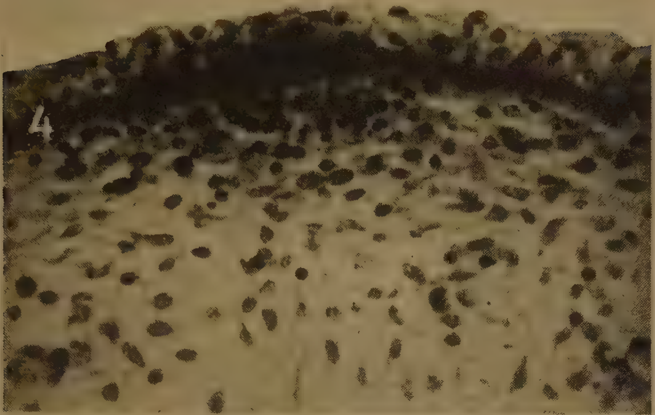
PLATE 2

EXPLANATION OF FIGURES

4 Section, 100-mm. fetus. Corrosive-acetic; hematoxylin and eosin. × 340. Proliferation of basal layer of epithelial cells. Beneath this there is condensation of mesenchyme. Moderate number of lymphocytes lying free in meshes of reticulum. In mesodermal nodules are several capillaries and mitotic figures.

5 Section, 160-mm. fetus. Corrosive-acetic; hematoxylin and eosin. × 340. Epithelial proliferation extends below surface level. Transforming of central epithelial cells into squamous type and sloughing of those at the surface. Mesodermal region is loaded with lymphocytes, many of which are undergoing mitosis. Epithelial and mesodermal portions distinct. There is a capillary in the nodule.

6 Section, 220-mm. fetus. Corrosive-acetic; hematoxylin and eosin. × 24. Transverse section through whole tonsillar region. Crypts, with branching. Some crypts in different stages of development. Many outgrowths cut transversely. Lymphoid tissue incloses epithelial tips. Mucous glands at sides of tonsil.



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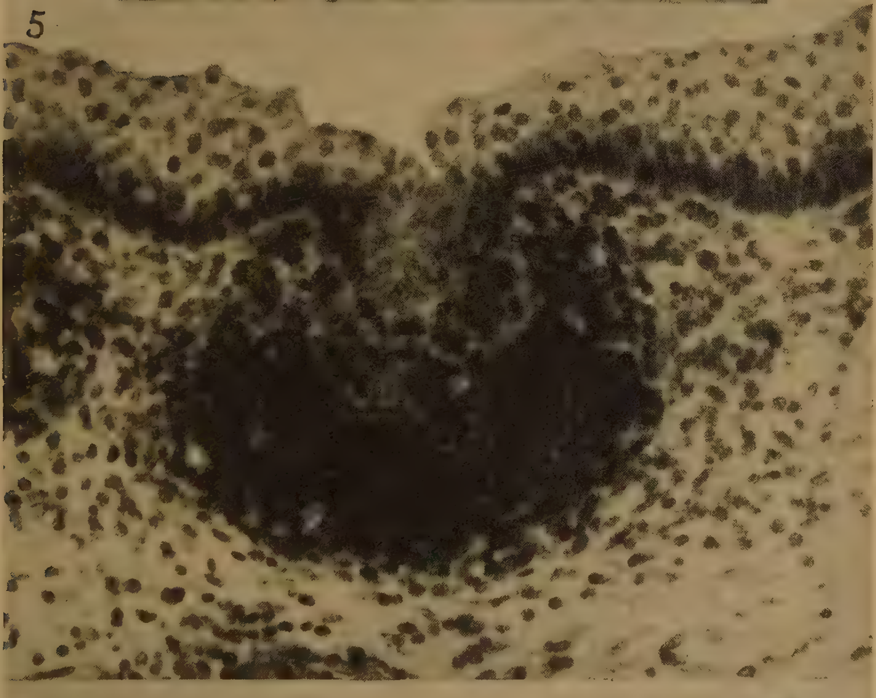

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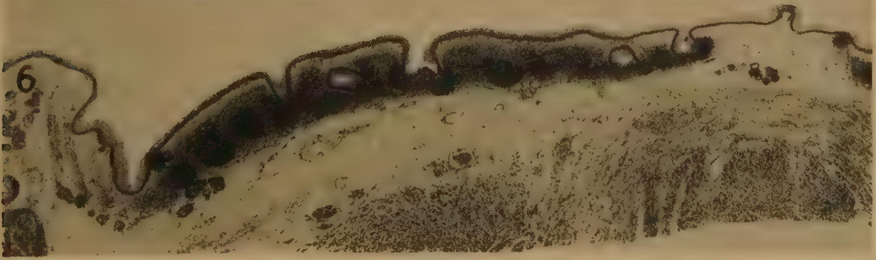

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NEW BOOKS AND PUBLICATIONS

NOTICES BY F. E. WELLS

ATLAS OF HUMAN HISTOLOGY AND MICROSCOPIC ANATOMY, by Johannes Sobotta. Edited from the fourth German edition by William Hunter Piersol. Volume 2, Atlas. Size, 8 × 9¾ inches. 92 pages consisting of explanations of the 535 illustrations on 68 colored and 24 uncolored plates, from original drawings by W. Freytag. Buckram. Price for the set of two volumes, $14.00. G. E. Stechert & Co., New York.

The translation of these books into English greatly widens their field of usefulness in this country. Only volume 2, the Atlas, has been published as yet. It is illustrated with the same plates, beautifully executed and reproduced, that made the fourth German edition noteworthy. Facing each plate is a page of explanatory print naming the structures shown, calling attention to important and significant features, and indicating briefly the technique used in preparation of material. Plates 1 and 2 show the individual cell, cell structure, and cell division. Plates 3 to 18 show the kinds of tissue—epithelial, connective, cartilage, endothelium, bone, blood, nervous, and muscular tissues. Plates 19 to 92 show the microscopic anatomy of the different organs. Volume 1 of the set, the textbook, will be issued in a few months.

DAS PRINZIP DER ANALOGIE UND DIE VERGLEICHENDE ANATOMIE, Eine Studie über eine Gesetzmässigkeit in der Biologie, by M. Nowikoff. 1930. Size, 6½ × 9½ inches. Pages iv + 185. 24 illustrations. Index. Price, RM 8. Gustav Fischer, Jena.

A comparative anatomy which emphasizes the similarity of form and function rather than a similarity of derivation and development. It comments on the mechanics governing animal growth and takes up in detail certain systems and organs: outer protective shells, central nervous system, eyes, luminary organs, and adjustments to locomotion.

PRAKTIKUM DER GEWEBEPFLEGE ODER EXPLANTATION BESONDERS DER GEWEBEZÜCHTUNG, by Rhoda Erdmann. Second edition. 1930. Size, 6¼ × 9¼ inches. Pages viii + 148. 99 illustrations. Bibliography. Index. Price, RM 14.80. Julius Springer, Berlin.

Tissue cultivation in vitro has made such progress in the last few years, and the technique has been so much simplified and improved by the introduction of heparin, the Carrel containers, and other discoveries that it was necessary for Doctor Erdmann to revise extensively the new edition of her book dealing with the theory and practical demonstrations of explantation and tissue culture. This book is designed for the use of university students and takes up all aspects of the subject, including the apparatus used, the best media, the behavior of various cells, and manifestations of growth.

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FORTY-SIXTH ANNUAL SESSION

University of Virginia, Charlottesville, Virginia

April 17 to 19, 1930

ABSTRACTS

PAPERS TO BE PRESENTED

1. *Some effects of removing endoderm from the stomodaeal region of Amblystoma punctatum embryos with closed neural folds.*

A. ELIZABETH ADAMS, Zoölogy Department, Mount Holyoke College.

After a transverse slit had been made in the ectoderm, a rectangle of endoderm was removed from the stomodaeal region of sixty-five Amblystoma embryos with closed neural folds. Thirty embryos were killed from three to twenty-two days after the operation. In most of these the wounds in ectoderm and endoderm closed, but regeneration of endoderm and contact of ectoderm and endoderm (the prerequisite for normal mouth formation) occurred relatively seldom. Tooth germs were found in fifteen animals old enough to have them definitely developed. Their arrangement was not normal and the numbers of both ectodermal and endodermal germs were markedly reduced, especially when there was no plug contact or open mouth. The reduction of tooth germs whose enamel organs are ordinarily derived from endoderm may be due to removal of endoderm at opera-

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tion and its failure to regenerate. The reduction in those with ectodermal enamel organs is more difficult to understand, since no ectoderm was removed. Other factors such as abnormal relationships in this region may be responsible, since in the few animals in which the mouth opened the arrangement and numbers tended more toward the normal condition. The bearing of these experiments on the rôle of epithelium (ectoderm or endoderm) and pulp (mesectoderm) in the development of the tooth germ will be discussed.

2. *Aberrant cells in the cerebellar cortex of the porpoise (Phocaena).* W. H. F. ADDISON, Department of Anatomy, University of Pennsylvania.

In addition to the typical cells forming the cortex of the cerebellum, there were seen in the porpoise conspicuous large cells scattered through the granular layer. These cells were present in both a young and an adult animal. Their shape was varied, but in general they were of two forms: 1) narrow, elongated, and, 2) stellate, multipolar. The dimensions of the former averaged 44 μ × 15 μ and of the latter, 41 μ × 23 μ. The Purkinje cell bodies measured 30 μ × 25 μ on the average. Thus in size they were comparable to the Purkinje cells. Their numbers, however, were much fewer, but they were constantly present, often two to three to a folium. They were present in all parts of the granular layer, often bordering on the central white substance of the folia. In Cajal silver preparations which showed the climbing fibers on the Purkinje cells, a corresponding fiber was occasionally seen on one of these cell bodies and running out one process. Similarly placed large cells have been reported in the cerebellar cortex of Elephas and Balaenoptera (Obersteiner, '13). They are probably Purkinje cells which have remained out of line with the other Purkinje cells during development, and have not been subjected to the same neurobiotactic force as the Purkinje cells, and hence have not assumed the usual form of these cells. They may be characteristic of the much-folded cerebellar cortex of the largest mammals.

3. *The effect of thymectomy on sexual maturity in the albino rat.* T. F. AHEARN AND T. T. JOB, Loyola University School of Medicine.

Technic and regulation of environment are important factors in such a question, but the relation of lymphoid tissue to metabolism is the main problem. Rats were thymectomized eighteen to twenty days postnatal. Sexual maturity of males was determined by presence of sperm in epididymis, females by mating. Early stock matings established the average sexual maturity at 76 days. females (range 65 to 98 days, one at 58 days). Postmortem examination of experimental animals 45 to 107 days. No effect in males. Sexual maturity in females average 57 days, range 46 to 66, or average advance of 19 days. This, associated with the fact that the human female is very much more subject to early sexual maturity in gonad disturbances, may be significant.

4. *Human ova from large follicles: including a search for maturation divisions and observations on atresia.* EDGAR ALLEN, J. P. PRATT, Q. U. NEWELL, AND L. J. BLAND, University of Missouri, Henry Ford Hospital, and Washington University School of Medicine.

Observations were made on number, size, and condition of large follicles in human ovaries removed at operation. The number of follicles ranged from two to thirty-two per ovary; the size, from 3 to 20 mm. Ova were recovered from follicles and sectioned separately.

Most of the normal ova were attached to the follicle walls. Mitotic division of follicle cells was used as a criterion of normal, growing follicles. The description of normal ova includes measurements and observations on living eggs checked by study of sectioned specimens.

One oocyte in the process of meiotic division was recovered from a 10-mm. follicle on the fourteenth day following onset of the previous menses. The chromosomes of the second maturation spindle were intact and twenty-two were counted. The first polar body contained twelve chromosomes. Seven follicle cells in mitotic division indicated that this follicle was still actively growing and might have ovulated. The pre-ovulation size of the human follicle is at least 10 mm.

A majority of large follicles were atretic and several interesting types of atresia were found. One type showed the invasion of follicle cells under the zona pellucida combined with vacuolation or fragmentation of the vitellus. This may be one source of 'cell balls' obtained by irrigating uterine tubes.

Selective elimination of ova occurs in human ovaries during late stages of growth of the follicles.

5. *The endolymphatic duct system in the two-year-old child.* BARRY J. ANSON AND JOHN GORDON WILSON, Departments of Anatomy and Otolaryngology, Northwestern University Medical School.

Models prepared by the Born wax-plate method from serial sections of the ear in a two-year-old child show that the utriculo-saccular and endolymphatic ducts in man, far from being simple tubular structures, actually possess striking regional complexities and, in the case of the latter duct, specialization of the epithelial cells similar to that described by Guild as occurring in the guinea-pig.

The lumen of the utriculo-saccular duct has the shape of an ellipse, greatly flattened in its transverse plane; the portion of the endolymphatic duct which joins the saccule is similarly compressed; the former duct, however, is greatly dilated at its point of communication with the utriculo-saccular duct; the dilatation becomes more capacious as it extends posteriorly. At a point opposite the junction of the utricle with the conjoined limb of the semicircular ducts, the endolymphatic becomes narrow again, as it turns superiorly, where, finally, it widens to join the endolymphatic sac. In the narrow portion of the duct and in the inferior part, at least, of the sac, longitudinal ridges of epithelium enclosing a connective-tissue core encroach upon the lumen of the tube; they are somewhat vascular, and seem to represent the projections seen by Guild, in sections, in the duct of the guinea-pig. There is some evidence, likewise, that their epithelial cells possess basal processes.

Photographs of the model will be exhibited (see Demonstrations).

6. *The sexual difference in the response of collateral circulation in the cat after ligation of the aorta at the lumbar level.* C. S. APGAR (introduced by Davenport Hooker), Department of Zoölogy, University of Pittsburgh.

By ligating the aortae of adult male and female cats between the adrenolumbar and iliolumbar arteries, it was found that there was a difference between the sexes in arterial collateral response. In the eight females operated, the average recovery time was 86 hours, with a range of from 72 hours to 120 hours, while the three males averaged 48 hours in their recovery time, with a range of from 24 hours to 72 hours. Though the number of cases studied thus far is small, it is believed that a marked difference exists in the experimental arterial collateral response described. This difference appears to be due to the inhibitory effect of the mammae and the ovaries in the female upon the collateral arteries in whose path they are situated.

7. *The relative volumes of the several lobes of the hypophysis cerebri in pregnancy.* WAYNE J. ATWELL, School of Medicine, University of Buffalo.

The relative volumes of the three epithelial lobes and the neural lobe of the hypophysis of the pregnant cat have been determined by the method of tracing every third section of the series on paper and then separating and weighing the several parts. There is no strikingly significant change in the relative volume of any of the epithelial lobes to accompany the progress of pregnancy. The conclusion seems clear that the enlargement of the hypophysis in pregnancy is due to hypertrophy or hyperplasia of all its lobes. This applies to the neural lobe as well as to the epithelial portions of the gland. Certain significant changes have been recorded for the anterior lobe proper and certain modifications for the pars tuberalis which may explain their contribution to the enlargement of the gland during pregnancy. The nature of the probable changes in the pars neuralis and in the pars intermedia have not been clarified.

8. *Osteogenesis of the human periotic capsule.* THEODORE HIERONYMUS BAST, Anatomical Laboratory, University of Wisconsin.

1. The bone of the periotic capsule is of three types: *a*) perichondral bone, *b*) endochondral bone, and, *c*) intrachondral bone or bone formed within the lacunae of the persistent calcified cartilage matrix.

2. The process of ossification of the cartilaginous capsule begins as follows: *a*) enlargement of the lacunae of cartilage; *b*) a modified staining reaction of the cartilage matrix; *c*) the entrance of connective-tissue cells into the enlarged lacunae which replace the cartilage cells; *d*) the entrance of vascular osteogenic buds which excavate large channels in this changed cartilage; this excavation is not complete, thus leaving islands of cartilage; *e*) primary endochondral bone is formed in the excavated parts; *f*) the connective-tissue cells which wandered into the lacunae of the islands of cartilage change into bone cells and surround themselves with bone; the cartilage matrix of these islands is calcified, thus producing a peculiar type of bone which has been designated as intrachondral bone, and, *g*) perichondral bone is formed around the original cartilaginous capsule.

9. *Contribution to the knowledge of the direction of flow of blood in the cardiac vessels.* O. V. BATSON AND S. BELLET, Graduate School of Medicine, University of Pennsylvania.

The direction of the flow of blood in vessels having openings in the right side of the heart was studied by injecting particulate matter into the veins of anesthetized, heparinized dogs. These particles have uniformly been found in the coronary sinus and the cardiac veins. The conditions of the experiments have been modified until they approach what we are pleased to call normal ranges of venous pressure. With the low pressure in the capillary bed, resulting from the fall in arterial pressure attending the experiments, the flow in the veins is toward the capillary bed. Considering the known rates of flow in veins and the sequence of contraction in the heart, these experiments indicate that the myocardium may be irrigated by an ebb and flow in the cardiac veins and give us a basis for understanding the persistence of life in reported cases of complete occlusion of the coronary arteries and in certain congenital anomalies.

10. *Neonatal decrease and recovery in thymus weight of man.* EDITH BOYD, Institute of Child Welfare and Department of Anatomy, University of Minnesota.

A statistical analysis of 500 necropsies of infants from birth to six months of age demonstrates that both the thymus weight and body weight for cadavera of given lengths decrease from birth to about three weeks and slowly increase thereafter. However, the body weight recovers sooner than the thymus weight. The thymus weight and body weight of stillborn infants of the same lengths were used as the basis for statistical comparisons. When the thymus weight for a given age is significantly below the stillborn thymus weight, the body weight for the same age is also significantly below the stillborn weight. Therefore ordinary unselected pathological material from birth to six months cannot be used to establish normal growth of the thymus gland, since these cases are usually both below length for age (premature) and below the weight of stillborns of the same length. When selected for good nutrition and accidental cause of death, the thymus increases in weight from about one month to fourteen years of age. The data meeting these criteria are rarely available in the first year of life.

11. *Reaction of the gall bladder to stimulation of visceral nerves.* EDWARD A. BOYDEN, Department of Anatomy, University of Alabama.

In a recent article (Am. Jour. Physiol., March, 1930) evidence was presented to show that both human and animal gall bladders are subject to the control of reflexes originating in the gastro-intestinal tract; that in unanaesthetized cats faradic stimulation of the empty stomach induces contraction of that organ and reflex emptying of the gall bladder; and that faradic stimulation of any part of the alimentary tube from stomach to caecum will inhibit the contraction of a gall bladder that is emptying after a meal of egg yolk.

Since then, employing similar methods (i.e., sewing insulated electrodes to various internal surfaces), these observations have been extended to the colon and rectum of the cat and to the peritoneal surface of the abdominal musculature, showing that faradic stimulation of that part of the gut tract or body wall that is supplied by sacral and thoracic nerves, respectively, also induces inhibition of the emptying gall bladder.

Using a modification of this method, by means of which electrodes may be wrapped around nerve trunks and insulated from surrounding parts, it has been found possible to stimulate the nerves of the unanaesthetized animal after its recovery from the operation and to demonstrate, in preliminary experiments, that direct stimulation of the intact hepatic plexus or of the plexus that accompanies the left gastric artery induces relaxation of the contracting gall bladder.

12. Some results following transplantation of the adult hypophysis into immature Amblystoma larvae. ROBERT K. BURNS, JR., Department of Anatomy, University of Rochester.

When the adult hypophysis is transplanted entire into young Amblystoma larvae 30 mm. in length, the following results occur: an intensification of the pigmentation verging upon absolute blackness; a somewhat accelerated growth over normal animals reared under identical conditions and with an identical ration; a tendency to metamorphose earlier than the controls; and a striking effect upon the male gonads, which are stimulated to enormous growth and to premature functional activity. In connection with the latter, effects are also seen on the secondary sex characters, which actually begin to appear in animals not yet metamorphosed, where virtually mature spermatozoa were present in the testes.

The same effects, to a slightly lesser degree, follow injection of an anterior-lobe extract. Further experiments are now under way involving separate transplantation of the parts of the hypophysis, although the results, with the exception of the pigmentation effect, are attributed to the anterior lobe.

13. The effects of x-rays on the chick embryo. ELMER G. BUTLER, Laboratory of Comparative Anatomy, Princeton University.

The effects of x-rays on the cells of the chick embryo at the end of two days of incubation have been studied in detail, with particular attention to the cells of the neural tube. Both lethal and non-lethal doses of x-rays have been utilized. The most obvious result of radiation is the complete disappearance within a very few minutes of all mitotic figures. However, it appears that all of those mitotic divisions which were in actual progress at the time of radiation have gone through to completion; the effect of radiation has been to prevent the initiation of further cell division. After the disappearance of mitotic figures, the nuclei remain in the resting state for several hours and gradually many of them enter a pycnotic condition. In those experiments in which non-lethal doses were used, the cells gradually recover, and finally, at the end of several hours, mitoses reappear. In those cases in which lethal doses were used, the cells never recover, more and more nuclei enter the pycnotic condition, and finally the cells go on to death and disintegration.

14. Growth in mass of the chick embryo and its membranes. THEODORE C. BYERLY (introduced by Frank A. Stromsten), Bureau of Animal Industry, U. S. Department of Agriculture.

Wet and dry weights of about 1600 embryos and their membranes have been obtained. All stages from unincubated blastoderms to hatched chicks, all of known parentage, were used. The instantaneous per cent growth-rate curve shows deviations from best smooth curve that are probably significant. During the first two days, experimental values lie below and from eleventh to fourteenth days above the smooth curve. The per cent dry weight of embryo falls until third day, then remains constant at about 5.5 per cent until tenth day; and from tenth to sixteenth rises to 18 to 19 per cent, the approximate value at hatching.

The allantois increases in wet weight from fourth to tenth day; decreases from tenth to fifteenth; then increases again until tenth-day value is regained. The dry weight of allantois reaches maximum on fifteenth day, then declines steadily up to hatching. The per cent dry weight of allantois also reaches maximum on fifteenth day and subsequently declines.

Growth in wet weight of yolk is composed of two distinct cycles. Increase in weight is almost at a standstill from seventh to tenth days, then increases rapidly to maximum on seventeenth, then declines up to hatching. The per cent dry weight reaches a maximum about the eleventh day.

15. *Experimental myogenic scoliosis.* EBEN J. CAREY, Marquette University School of Medicine.

The prevalent theory regarding the etiology of rotary lateral deviation of the human spine is the influence of the dead static load of body weight on the upright spinal column. The time of appearance, localization, and direction of the process of ossification of the arches and bodies, respectively, of the vertebrae closely parallel the appearance of muscular activity that compresses the spine through direct or indirect muscular attachments.

The spine is tentatively considered as the skeletal indicator of a bilaterally symmetrical muscle scale. The deviation of the spinal indicator to the left or to the right is directly related to muscle imbalance. Evidence has been obtained on over 150 young guinea-pigs, rats, and dogs, through selective muscular excisions, localized destruction of nerve supply to muscle, experimental herniae, amputations of limbs and suturing the tails to scapulae of white rats, and unilateral blindness, that not only the functional, but the structural rotary lateral scoliosis as well, with both primary and compensatory curvatures, of an S-shaped character, may be produced and the time factor subsequent to the experiment. The experimental evidence points to the conclusions that structural rotary scoliosis may be a deformity of bone growth the resultant of imbalance of the bilaterally symmetrical musculature of the body and to the importance of nerve and muscle conservation in any operative procedure on the growing organism.

16. *Some effects of parathyroidectomy on pregnancy and the sex ratio in albino rats.* S. B. CHANDLER (introduced by R. M. Strong), Loyola University School of Medicine.

To study the effects of parathyroidectomy on pregnancy and the sex ratio in the albino rat, these experiments have been done. Twenty-five pairs of normal stock rats were mated and allowed to bear one or two litters. This group of control litters contained 333 rats, with a sex ratio of 156 males and 177 females. Following a short rest period, both parents were parathyroidectomized.

Females become pregnant probably as regularly after operation as before. Four died near term. Since no full-sized litters with all members living could be obtained, the following procedure was adopted. Near expected delivery, provided the condition of the animal would permit, the uterus was incised and the fetus removed. This group constituted eleven litters containing 172 animals with a sex ratio of ninety-one males and eighty-one females. In a second series the parents were parathyroidectomized at sixty-five days and mated at ninety-five days. Five litters containing fifty-two animals have been obtained by operation in this series.

The number per litter is about the same after operation as before. Twelve females have been opened one to three times with only one death. All the symptoms of acute tetany and depression disappear when the uterus is emptied. Recovery is as rapid as in normal pregnant animals subjected to an abdominal opening.

17. *The histology of the anterior pituitary of the albino rat in relation to the oestrous cycle.* Harry A. Charipper and H. O. Haterius (introduced by Robert Chambers), Department of Biology, New York University.

Recent investigations of the anterior pituitary-ovarian interrelationship seem to indicate a functional rhythmicity of the anterior pituitary at least insofar as the nature of the oestrous cycle in the albino rat is concerned. That such a possible rhythmicity might be reflected in the histological picture did not seem unlikely. Accordingly, an examination has been made of serial sections of the anterior lobe taken from animals in different phases of the oestrous cycle. Striking histological differences are displayed. The pars anterior of litter mates in late dioestrus shows the presence of basophiles and eosinophiles, with eosinophiles in dominance. The pars anterior of litter mates in late oestrus shows the presence of basophiles, with occasional poorly staining eosinophiles. Further, the basophiles in the pituitary of late dioestrus show a compact nucleus surrounded by a meager amount of light blue cytoplasm, whereas the basophiles in the late-oestrus pituitary show a compact nucleus surrounded by a goodly amount of light blue cytoplasm. It appears possible, therefore, that there is a histological rhythmicity of the pars anterior of the pituitary gland which may be correlated with the oestrous cycle.

18. *On the termination of the circumflex veins of the thigh.* C. M. Charles, T. L. Finley, R. D. Baird, and J. S. Cope (introduced by R. J. Terry), Department of Anatomy, Washington University, St. Louis.

The mode of termination of the medial and lateral femoral circumflex veins of the thigh was observed in fifty-three white male and thirty American negro male cadavers. In the modern text-books of anatomy the circumflex veins are commonly described as terminating in the deep femoral vein. Only 1.2 per cent of all the cases observed were of this type. In 81 per cent of the bodies, on both the right and left sides, the medial and lateral femoral circumflex veins terminate in the femoral vein. The lateral femoral circumflex vein is represented by two vessels in 19 per cent of the cases. The observations on this material confirm the earlier description of Theile and the more recent results of Picque and Pigache. A definite tendency is present for the veins to terminate at a level proximal to that of the origin of the arteries they accompany.

No marked differences exist between the two sides of the body in the manner of termination of the veins. Regarding racial comparison, these veins in the American negro males are probably less variable in their termination than are those of white males.

19. Studies of the brain of Bregmaceros. H. H. CHARLTON, Department of Anatomy, University of Missouri.

This specimen was collected by the Bingham Oceanographic Expedition at 800 fathoms. The rostral end of the spinal cord shows an enlargement of the dorsal columns forming five lobes. The four caudalmost lobes receive a small dorsal root from the first spinal, but enormous dorsal roots from the second, third, and fourth, and seem related to a group of filaments, possibly tactile organs, which extend from the mouth region nearly to the tail.

This reminds one of the condition in the sea robin, only the increase in size of the dorsal columns is more pronounced in Bregmaceros. Furthermore, the secondary tracts from the enlarged areas, instead of being adapted only for short local reflexes as in Prionotus, ascend by both uncrossed and crossed tracts. The latter tract, the spino- and trigemino-mesencephalicus, reaches to the optic tectum and is by all odds the most prominent tract in this brain. From it many fibers are given off to the reticular nuclei.

In addition to the dorsal and ventral roots, each of the first four segments possesses an additional root which usually arises just caudal to the emerging ventral roots. This third root which suggests the sympathetic passes dorsally and slightly caudalward inside the cranium, finally piercing it and joining a ganglion lying outside the cranium and dorsolateral to the spinal cord.

20. Morphology of the cranial autonomic neurons. KERMIT CHRISTENSEN (introduced by Albert Kuntz), St. Louis University School of Medicine.

Preparations of the cranial autonomic ganglia (cat, dog) were studied with especial reference to the general morphology of the neurons and the character and distribution of their processes. The neurons in the several cranial autonomic ganglia are compared. The cranial autonomic neurons are also compared with sympathetic neurons, particularly those of the ganglia of the sympathetic trunk.

21 a. Observations on living preformed blood vessels as seen in a transparent chamber inserted into the rabbit's ear. b. Arteriovenous anastomoses. E. R. CLARK AND ELEANOR LINTON CLARK, Department of Anatomy, University of Pennsylvania.

a. By means of a modification of the method described by Sandison, which was worked out in this laboratory, for the study of new-formed tissue, it has been possible to retain in a transparent chamber preformed tissue, including the main artery of the ear and a large area of arteries, veins, and capillaries, which can be studied continuously in the living animal for many weeks. This method allows a precise study of the morphology and physiology of the original vessels of the ear and their comparison with the newly formed vessels seen in the other type of chamber. A description is given of some of the observations which have been made, including the contractions of arteries and arterioles and the changes concerned in the adjustment to the new environment as seen in daily observations and frequent photographic records, for several weeks beginning immediately after the insertion of the chamber.

b. Arteriovenous anastomoses are present in large numbers in the rabbit's ear. Preliminary studies have been made upon them, as seen in the 'preformed-tissue' chamber, by daily observations extending over a period of weeks. Their formation has also been observed in chambers with 'new-formed' tissue.

22. *The vascularity of the hippocampal cortex in the rat.* E. HORNE CRAIGIE, University of Toronto.

In both fascia dentata and hippocampus proper the molecular layer is markedly the richest in capillaries, surpassing also the corresponding layer in the neocortex. The other archicortical laminae are poor as compared with the neocortex, the stratum granularis of the dentate fascia being particularly poor, and the strata pyramidalis, oriens, and radiatum ranking in that order in the hippocampus proper. In a general way the archicortex ranks in vascularity with the poorest sensory and correlation centers.

On account of the difference in relative vascularity of the molecular layer in archi- and neocortex, the average value for all layers should not be used for comparison. In the neocortex at twenty-one days of age the relative vascularity of the molecular layer is similar to that in the adult archicortex, and it progressively loses, in comparison with the other layers, between that age and maturity.

Measurements in the corresponding parts of a series of wild Norway rat brains have given in all cases figures below the average for albinos.

23. *Morphological evidence of physiological activity in blood cells.* R. S. CUNNINGHAM, Department of Anatomy, Vanderbilt University School of Medicine.

In studying living white blood cells, stained supravitally with neutral red, certain characteristics have been observed which indicate to some extent the physiological state of these cells. These characteristics are the size, color, distribution, and rate of formation of vacuoles which stain with neutral red. Normal granulocytes do not contain vacuoles after twenty minutes' staining, but after thirty to forty minutes they appear and subsequently increase in size and number. Granulocytes, from patients having pyogenic infections, show vacuoles after ten to twenty minutes' staining. It seems possible that the formation of vacuoles in normal cells results from the accumulation of waste products, the abnormal environment being the stimulus. The cells from patients with pyogenic infections probably undergo a similar change in the blood stream. Normal monocytes show vacuoles of small and medium size, which are brown in color when stained for fifteen to twenty minutes. On standing, these increase in size and number, change from brown to red, and eventually coalesce. Under certain conditions (e.g., tuberculosis) the vacuoles of the monocytes increase in number, but decrease in size. In other conditions (e.g., liver injuries) monocytes develop much larger vacuoles. It seems probable that the larger size of the vacuoles is due, as in the granulocytes, to some stimulating factor. The fine diffuse granulation, on the other hand, suggests a depressed metabolic state.

24. *A topographic study of the red nucleus and adjacent cell groups in the cat and rabbit.* H. A. DAVENPORT AND S. W. RANSON, Northwestern University Medical School.

A reconstruction of the distribution of the large cells of the red nucleus in cat and rabbit showed a striking similarity in the two animals.

No definite small-celled rostral division was seen, but the small ganglion cells were found to be most numerous near the center of the nucleus in the region adjacent to the rostral fibers of the root of the third nerve.

The red nucleus can be conveniently divided into a compact (caudal) and a diffuse (rostral) part.

Other nuclei of the tegmentum and subthalamus occupy approximately the same levels in the two species, hence any difference in behavior noted after transection of the brain stem at the same level in each is not explained by anatomical differences.

25. Mitochondria and neutral-red bodies in the differentiating erythrocyte of Necturus. Alden B. Dawson, Zoölogical Laboratory, Harvard University.

Practically all stages of the maturation of the erythrocyte are encountered in the peripheral blood of Necturus during the intense regeneration which occurs following the secondary anemia produced by lead poisoning. Supravital staining methods were employed. The youngest stages, containing but a trace of hemoglobin, possess large rod-shaped mitochondria scattered diffusely through the cytoplasm. The neutral-red bodies are few, minute, and uniformly distributed. In cells possessing more hemoglobin, the mitochondria are always greatly increased in number and are either in the form of large or small granules. The larger mitochondria are frequently loosely grouped about the nucleus, while the smaller, granular forms are diffusely scattered. The neutral-red bodies are still minute, slightly increased in number, and uniformly distributed through the cytoplasm. In the mature cell, however, the mitochondria are strikingly reduced in number and occupy a perinuclear position. They are in the form of relatively long, beaded filaments. The neutral-red bodies are not increased in number, but are larger and definitely grouped as two bipolar clusters. Many of the younger cells exhibit active mitosis and it appears that the large mitochondria may be associated with the growth phase, while the small granular forms indicate a phase of cellular proliferation. The restricted distribution in the mature cell of both mitochondria and neutral-red bodies is probably associated with the assumption of the typical form.

26. The mode of row formation of cartilage cells in endochondral ossification. G. S. Dodds, Medical School, West Virginia University.

This process has been studied in embryos and young animals of dog, cat, pig, and man, revealing the following sequence of events. At a definite distance from the advancing ossification front the cartilage cells begin to divide in a different manner from those at a greater distance from it; here each dividing cell gives rise to two flattened cells. These pairs of cells are the beginnings of rows of cells which are formed from them by repeated divisions, the cells remaining flattened and of circular outline until the full number in each row has been produced. When the full number, which is rather constant in any one front, has been produced, the cells enlarge greatly and undergo no further divisions. The divisions of the circular, flattened cells in the rows take place in a direction transverse to the long axis of the rows of cells. Each cell thus gives rise to two semicircular cells, each of which soon becomes circular by the growth of a wedge-shaped extension past its mate.

27. *The relation of cerebrum, cerebellum, brain-stem, and encephalon weight to body length throughout fetal and postnatal life.* HALBERT L. DUNN, The Mayo Clinic, Rochester, Minnesota.

The function $Y = \frac{K}{1 + ae^{bx} + ce^{dx}}$, in which Y is the brain or brain-part weight; x, stature; and a, b, v, d, and K, empirically determined constants, has been fitted to total brain, cerebrum, cerebellum, and brain-stem weight. This function is the only one yet found by which these data can be expressed in terms of stature throughout the entire fetal and postnatal periods and which also fits the observations within the variation expected by sampling. K represents the asymptote of the adult value, b and d are 'true' rate constants, b representing the rate of 'dampening' on growth and d, the acceleration.

Early in fetal life the cerebrum and cerebellum grow at the same relative rate and the brain stem about two-thirds as fast. The cerebellum maintains its relative rate fairly well to birth. The cerebrum and brain stem decelerate steadily. By birth they are growing at the same relative pace, while the cerebellum is increasing at almost twice this relative rate. Thereafter the cerebellum decelerates very rapidly. At one year, all three brain parts are again growing at about the same relative rates (less than one-third of the relative speed of early fetal life). After this the growth rate in cerebrum and cerebellum rapidly becomes negligible, while the brain stem continues to grow at a decelerating pace to about five years.

28. *The renal tubule and glomerulus.* J. GRAHAM EDWARDS, Department of Anatomy, School of Medicine, University of Buffalo.

Dyes and iron salts injected into frogs at various intervals prior to anaesthesia and exposure of the kidney for microscopic observation are not concentrated in the lumen of the tubule until the heart beat is reduced in number per minute or in strength, with an accompanying slow renal circulation, inactive glomeruli, and slight to no urine elimination by the kidney. Iron salts, though not visibly concentrated like dyes in the living kidney, are seen to be comparably concentrated when at comparable stages the kidney is fixed in situ by trichloracetic or hydrochloric acid. Subsequent maceration reveals the precise locus within the tubule where the iron is luminally more concentrated. This locus is the intermediate segment and distal convolution.

That active glomeruli are essential in masking or preventing dye and salt concentration in the segments mentioned is shown by precise inactivation in diuretic kidneys of the glomerulus alone and observing the same picture of dye concentration only in the tubule with which the inactivated glomerulus is connected—similarly, if a minute portion of a proximal convolution is blocked. This relation of glomerulus to tubule and the contingent phenomenon of luminal concentration affords a basis for interpreting the significance of the alternation in glomerular activity established for the mammalian kidney by Hayman and Starr ('25).

29 a. Some effects on the hypophysis of hyper- and hypothyroidism. Herbert M. Evans and Miriam E. Simpson, Anatomical Laboratory, University of California.

Young adult female rats (150) were chosen so that we had three sisters from each of fifty litters. One group was submitted to hyperthyroidism. Each animal received daily 1 gram of fresh beef thyroid. In another group of sisters the thyroid was ablated. The third group served for normal controls.

At the end of five weeks, the hypophyses of these animals were implanted into immature females to discover whether any change had resulted in the power of the hypophysis to provoke sexual maturity. Two glandules were implanted daily for three days and the young recipients sacrificed four days after beginning the experiment.

The animals fed thyroid possessed lighter hypophyses (8.8 mg.) than their normal sisters (10 mg.), whereas the thyroidectomized sisters possessed heavier hypophyses (11 mg.). The hypophyses of hyperthyroidized animals, although smaller, are more effective, the hypophyses from thyroidectomized animals, although larger, are less effective in provoking precocious sexual maturity than are the hypophyses of normal animals.

Since the thyroidectomized donors were seriously stunted in their growth, the increased absolute weight of their hypophyses constituted a very considerable relative increase, and the decrease in its power to provoke sexual ripening in young recipients is all the more striking. It would seem fair to deduce direct impairment of this hypophyseal function in the absence of the thyroid.

29 b. Different effects secured from intraperitoneal as contrasted with subcutaneous administration of the anterior-hypophyseal hormones. Herbert M. Evans and Miriam E. Simpson, Anatomical Laboratory, University of California.

When aqueous extracts of the placenta are prepared according to the method of Wiesner and injected subcutaneously (1 cc. daily for three days) into immature female rats, almost all animals (twenty in twenty-two) showed rupture of the closing membrane and the oestrous smear at the end of the fourth day, whereas with intraperitoneal dosage practically none of them (only two in twenty-two) exhibited these effects, though in both groups corpora lutea were the rule.

Extracts produced by treating Prolan with sulphosalicylic acid, when injected in these two ways, also gave these strikingly different results. The subcutaneous dosage led to rupture of the membrane, the cornified smear, and normal corpora lutea, the eggs being encountered in the tube at four days, but the groups receiving intraperitoneal dosage did not show opening of the vagina even by the eighth day when sacrificed. The vaginal epithelium was that of pseudopregnancy and the ovaries possessed corpora with inclosed eggs.

These facts support strongly the assumption of two anterior-hypophyseal sex hormones, one stimulating follicular growth and rupture, and the other, precocious luteinization. If extracts like the above consisting chiefly of the follicle-stimulating hormone be administered intraperitoneally, detection of their chief constituent will be impossible. On the other hand, intraperitoneal administration in essential for the detection of traces of the luteinizing hormone.

29 c. *Subnormal sex-hormone content of the hypophysis of animals with inadequate antineuritic vitamin B.* HERBERT M. EVANS AND MIRIAM E. SIMPSON, Anatomical Laboratory, University of California.

Evans and Bishop showed that females suffering from inadequate antineuritic vitamin B ovulate infrequently, if at all. This ovarian delinquency can be referred to injury to the hypophysis by two lines of evidence: first, the implantation of normal hypophyseal substance into such females provokes follicular ripening, oestrus, and ovulation; secondly, the hypophyses of animals suffering from inadequate antineuritic vitamin can be directly shown to be deficient in their possession of the sex hormone. The first type of experiment has been done repeatedly by us and has also recently been published by A. S. Parkes. The second type of experiment was done as follows: males were taken at weaning and fed a diet adequate save in the antineuritic vitamin for about forty days. Their litter-mate brothers were on the same diet with adequate amounts of this vitamin. Stunting resulted in the defective-vitamin group. As there was this discrepancy in body weight, another group of controls was sought in younger animals of the same body weight as these defective ones. All groups were sacrificed at forty days and their hypophyses implanted into immature females. The hypophyses in animals held on inadequate antineuritic vitamin are markedly defective in their power to evoke precocious maturity when compared either with the hypophyses of their adequately fed brother controls or the glands from younger animals of their same body weight.

30. *The origin of asymmetry in the egg of Triton as shown by the artificial production of situs inversus viscerum.* G. FANKHAUSER (introduced by F. R. Lillie), Department of Zoölogy, University of Chicago.

Fertilized eggs of the newt Triton palmatus were cut in halves by means of a hairloop (Spemann's method). The half containing the diploid fusion nucleus may develop into either perfectly normal or strikingly asymmetrical larvae. In the latter the defective side always corresponds to the original inner side of the egg-half. If a larva has a defective left and a normal right side, it is called a 'right' larva and interpreted as having originated from an egg-half which in the normal development of the whole egg would form the right side of the embryo. A corresponding explanation is given to the origin of a 'left' larva. This shows that the egg, at the time of operation, has already a bilateral symmetry.

The situs viscerum may be easily seen in living larvae. It was normal in all symmetrical larvae (fourteen cases) and in all 'left' larvae (six cases). Of the ten 'right' larvae, however, five had situs inversus of the digestive tract and the heart and five were normal.

These results agree with those obtained by Spemann in his experiments on cleavage stages and confirm his conclusion that the typical asymmetry is established in the unsegmented egg. Mangold's suggestion that it arises as an asymmetry of the center of organization would also be consistent with my results, this center being present shortly after fertilization.

31. *Elastic tissue; its phylogeny and ontogeny in vertebrates.* S. H. GAGE, Cornell University, Ithaca.

In the vertebrate series, elastic tissue first appears in the elasmobranchs. It seems to be wholly absent from Amphioxus, Myxine, and the petromyzonts.

In the shark, chimaera, ganoids, teleosts, amphibia, reptiles, birds, and mammals, it is abundant in the arterial system. It is scanty or absent in the veins and connective tissue generally of the lower forms. With the amphibia, and onward in the scale, it increases in amount in the veins and fibrous tissue generally until in adult mammals nearly all of the fibrous tissue of the body contains a greater or less amount. It is always associated with white fibrous tissue even in the ligamentum nuchae of ruminants.

In ontogeny, the elastic tissue first appears in the arteries. Mall found it in the aorta of a 40-mm. pig.

As development proceeds the elastic tissue increases in the arteries and appears in the veins and the connective tissue generally.

Its definite function is to retract after being stretched, and it appears in the development of the different organs only shortly before it is needed.

32. *Differentiation of mitochondria and granules by microdissection.* C. M. GOSS, Department of Anatomy, College of Physicians and Surgeons, Columbia University.

In order to compare mitochondria and granules experimentally, two cells from the circulating blood, lymphocytes and polymorphonuclear neutrophiles, were selected, because the former contain many mitochondria and only one or two granules, while the latter are filled with granules and seldom contain mitochondria. Living cells were studied at body temperature in plasma, serum, Ringer solution, and media with altered osmotic pressure and pH. By dissecting the cell with microneedles, granules were separated from the cytoplasmic matrix and were observed in brownian movement both inside and outside the cell. The mitochondria could not be released from their combination with the matrix in this way. These results suggest that granules are discrete particles suspended in the matrix, but that mitochondria are more in the nature of specialized or condensed areas within the cytoplasm proper.

33 a. *A gross cochlear anomaly, scala communis cochleae, in adult with normal hearing; the bearing of this fact on theories of hearing.* STACY R. GUILD, Otological Research Laboratory, Johns Hopkins University.

There is a large opening in the bony septum between the lower half of the middle turn and the apical turn of the cochlea in the one ear obtained of a colored man thirty-eight years old. Four weeks before death, his hearing acuity was fully normal for all octave tones from 64 to 8192 double vibrations per second. The anomaly is similar to that in the infant's ears described before this association last year in connection with a discussion of its embryological significance. In neither case is it associated with obvious defects of the nerve, such as have been found in most of the dozen or so other cases reported in the literature.

In several of the theories of hearing which have been proposed one of the essential assumptions is that sound waves in the perilymph pass 'up' along the scala vestibuli to the helicotrema and then 'down' the scala tympani. The large opening present in this cochlea would permit such sound waves to 'by-pass' below the helicotrema; and this should, if any of these theories are correct, have caused such interference of wave systems as to have seriously interfered with hearing, at least for low tones. Actually, the threshold for low tones was slightly better than average. There are no data on his ability to discriminate between the pitch of different tones.

33 b. The control of gas-bubble formation in tissue cavities during histologic preparation. STACY R. GUILD, Otological Research Laboratory, Johns Hopkins University.

Utilizing the indications given by the study of gas accumulation in immersed closed collodion sacs (Guild, Science, September 20, 1929) many human temporal bones have been prepared, the sections of which are quite uniformly free from the serious gas-bubble distortions of the membranous labyrinth which have been such an exceedingly frequent source of trouble for every investigator of the inner ear. The essential variation applied to each method tried has been the reduction of the dissolved air content (to well below saturation) of as many as possible of the fluids used, employing faucet filter-pump evacuation immediately before use. (With acid or formalin mixtures the 'degassing' is done before adding these components.)

It is evident that the phenomena originally observed are not peculiar to collodion semipermeable membranes. The principle is applicable to many other problems.

The faucet filter-pump method of 'degassing' the amount of water needed in my laboratory for washing out fixing fluids and acids from temporal bones was so time-consuming that, after the principle had proved its usefulness, I designed and had constructed an apparatus which delivers a continuous supply of almost entirely gas-free water directly into the specimen containers. The gases are removed by boiling as the water passes through an elongated shallow tank from which it is conducted through a cooling coil to suitable outlets.

34. Parturition in the monkey (Macacus rhesus). CARL G. HARTMAN AND O. L. TINKLEPAUGH, Carnegie Laboratory, Baltimore, and Institute of Psychology, Yale University.

The period of gestation having been determined previously to be about six lunar months, it was relatively easy to observe and photograph parturient female macaques in the Carnegie colony in the spring of 1929. During May and June eleven rhesus babies were born and six deliveries witnessed, which brings our total observed and recorded births to seven. Photographic records were secured of six deliveries in the form of snap-shots or motion pictures or both. The remaining five subjects were seen before and after the actual delivery. Labor usually lasts about one and one-half hours (fifteen minutes and thirty-four hours are the extremes). The female generally assumes the squatting posture. She assists in the delivery by manual efforts, pulling the baby forth as soon as she can grasp it. In adaptation to arboreal life the baby takes hold of the mother's skin, even before it is completely born. The mother licks the baby, her own hands, even the floor with greater or less thoroughness. In all normal births but one the afterbirth was eaten ravenously down to the cord, which dries and drops off in about twelve hours. No outcry was uttered in any of the seven cases. From these and Spiegel's single case it is possible to construct for the first time a pretty definite behavior pattern for the birth processes in the monkey.

35. A method for the correlation of cell types with fiber types in the autonomic and somatic nervous systems. PETER HEINBECKER AND JAMES L. O'LEARY, Departments of Surgery and Anatomy, Washington University, St. Louis.

Investigations by Heinbecker ('29) and Bishop and Heinbecker ('30) have provided a means of distinguishing fiber types in autonomic and somatic nerves. Because of the extreme rapidity of the cathode-ray oscillograph as a recording mechanism, it is possible to differentiate three to four components of potential. By a correlation of the fiber types revealed in cross-sections of nerves with their conducted action potentials, it is possible to assign to each component of the electroneurogram a definite fiber type. Thresholds and maxima of the fiber types vary to a degree which permits them to be stimulated separately.

Prolonged repetitive stimulation of the axon brings about granulation of the chromatin and its clumping about the nucleolus. The chromatin mass resembles the nuclear inclusions which have been so much discussed as pathognomonic of several virus diseases.

The nuclear change described has been observed: in autonomic ganglia following pre- and postganglionic stimulation, in spinal ganglia after stimulation of the dorsal root, in anterior-horn cells following stimulation of the cord, and in ganglion cells of the heart following stimulation of the vagus. In autonomic nerves, the fiber groups have been differentially stimulated, with the result that only those ganglion cells belonging to or synapsing with them showed the characteristic nuclear alterations. The widespread applicability of this method for demonstrating the cells belonging to the various fiber types is therefore apparent.

The experiments were conducted in vitro, upon nerves from the turtle, cat, and monkey.

36. A presomite human embryo with a germ disk measuring about 1.6 × 0.9 mm. CHESTER H. HEUSER, Department of Embryology, Carnegie Institution of Washington, Baltimore.

The chorion was removed intact from a uterus which had been cut open and fixed in formalin. A small symmetrical embryo, with a germ disk 1.6 × 0.9 mm., was found attached to the chorionic wall. The yolk sac measures about 1.5 × 1.1 mm. Through the evenly distended amnion, which is of small size, the form of the embryo can be plainly seen. The embryonic area is oval in outline, tapering toward the body stalk. The primitive node of Hensen and the notochord stand out with diagrammatic clearness in the intact embryo. These structures are plainly visible through the amnion and they are especially clear in the ventral view, where they can be studied through a tear in the yolk sac.

A small detached cyst, found some distance from the embryo, measuring 0.63 mm. in diameter, probably represents a cut-off portion of the distal pole of the yolk sac where a drawn-out process is commonly found.

The stage of development of the embryo indicates that it should be placed in a group which includes the specimens described by Frassi, Thompson, and Brash, Grosser (Kl), Bryce (McIntyre), and v. Spee (Glaevecke).

The well-fixed and transparent tissues permitted us to secure good photographs of the embryo: these will be shown in lantern slides.

37. *Restoration of the liver following partial hepatectomy in white rats.* G. M. Higgins and R. M. Anderson, University of Minnesota.

This study of the degree and rate of compensatory hyperplasia of the liver, following partial removal of the organ, has been undertaken to determine if possible the morphologic and physiologic factors incident to restoration. Under ether anesthesia, 50 to 75 per cent of the liver has been removed from a large number of animals and daily observations have been made upon the general health, body weight, and degree of hepatic compensation for a period of six weeks, when restoration is ordinarily complete. Wet and dry weights of the liver, together with volume readings, have been made at frequent intervals during the period of restoration. Regeneration from the level of the cut does not take place; but restoration is effected by a hyperplasia of remaining intact lobes.

In addition to a study of the ensuing hyperplasia following a simple partial hepatectomy, studies have likewise been made upon the degree and rate of restoration in animals in which, *a*) the common duct was doubly ligated and sectioned prior to the removal of the usual amount of liver substance, *b*) the portal vein had been partially occluded prior to removal, to restrict the portal blood supply to the liver remnant, *c*) the reticulo-endothelial cells in the liver had been partially 'blocked' prior to partial removal.

38. *Studies in the innervation of skeletal muscle. III. The extra-ocular muscles in the rabbit.* Marion Hines, Department of Anatomy, Johns Hopkins University.

Studied with the methylene-blue-injection method, the four morphologically distinct types of nerve endings, other than those found on blood vessels, in the extrinsic eye musculature of the rabbit are: terminaissons en grappe, naked endings, accessory hypolemmal terminations, and motor end plates. The terminaissons en grappe originate from heavily medullated nerve fibers, distributed longitudinally to the muscle fibers; from non-medullated nerve fibers, which traverse the muscle fibers; and from non-medullated axis cylinders which share in common with the motor end plate a medullated nerve fiber, similar to the manner of origin of the accessory hypolemmal termination. The naked endings are branches of medullated or continuations of non-medullated nerve fibers.

After removal of both superior and middle cervical ganglia together with their connecting chain, these endings remain undisturbed, as do, sometimes, nerve fibers about the larger vessels. When the oculomotor nerve is severed at the base of the brain, only nerves supplying blood vessels remain. Following a combination of those two operative procedures, all nerve fibers are lost or in an advanced stage of degeneration. It is apparent, then, that the extravascular innervations of the four muscles involved are supplied by the oculomotor nerve. The blood vessels are not always completely denuded by sympathectomy alone, but in the writer's experience such nerve fibers are completely lacking after sympathectomy and cutting the third nerve.

39. Responses elicited by stimulation of the mesencephalic tegmentum in the cat. J. C. HINSEY AND S. W. RANSON, Northwestern University Medical School.

The mesencephalic tegmentum was explored in the cat, using unipolar and bipolar stimulation, in an analysis of the responses which have been described by Thiele ('05), Graham Brown ('13, '14, '15), Weed ('14), and Környey ('27). The tegmental response elicited by stimulation of the mesencephalic tegmentum in the region of the red nucleus in the cat is characterized by ipsolateral flexion and contralateral extension in the fore limbs and varying responses in the hind limbs. The ipsolateral flexion relaxed quickly on cessation of the stimulation, while the contralateral extension was followed by a tonic afterdischarge. In these experiments, the tegmental response was found to occur following section of the decussation of Forel, bilateral section of the dorsal roots of the first three cervical nerves, removal of the cerebellum and the substantia nigra, and bilateral section of the medial longitudinal fasciculi. It was not found to occur on stimulation of the tectum or on the ipsolateral side following hemisection caudal to the red nucleus and the decussation of Forel. These observations together with the other evidence suggest that the pathway is ipsolateral down into the reticular formation from which it may be mediated over the reticulospinal tracts. Stimulation of the basis pedunculi in the region of the corticospinal tracts produced contralateral flexion in the fore and hind limbs.

40. Rhythmical and arhythmical secretion. G. C. HIRSCH (introduced by E. V. Cowdry), Utrecht (Holland).

It is possible to divide the happenings during secretion into four subdivisions: 1) reception of water and materials (which can occur singly in the pancreas, but generally together in Astacus); 2) formation of prosecretion, occurring in the pancreas at the base of the cell (observations of living cells); 3) elaboration and migration of enzyme granules to the apex of the cell; 4) extrusion of either liquid enzyme, granules, large parts of cells (Helix), or of entire cells (Astacus). In the pancreas the extrusion is aided by many large water vacuoles.

These four processes happen repeatedly in polyphasic cells (pancreas, Helix), but only once in monophasic cells (Astacus); in both types there is, however, a restitution of lost plasma and enzymes; in the former, through the cell base (pancreas); in the latter, by mitosis of embryonal cells.

Restitution is a factor in rhythmical and arhythmical secretion. If the time of restitution equals the time of extrusion, there is an arhythmical secretion of the cell or gland. If the time of restitution is longer than that of extrusion, the result is a rhythmical secretion of cell or gland. Thus far the following systems have been found: 1) mouse pancreas, cells rhythmical, organ arhythmical; 2) Helix, cells rhythmical, organ rhythmical because the cell activities are synchronized; 3) Astacus, cell arhythmical, organ rhythmical since the mitosis is rhythmical.

41. *The formation of the fasciculus longitudinalis medialis in embryos of the albino rat.* IRA D. HOGG, University of Pittsburgh and The Wistar Institute of Anatomy and Biology.

The first neuroblasts to differentiate in embryos of the albino rat lie just rostral to the cephalic flexure. Toward the close of the eleventh day of gestation, the axones of these cells grow caudad, forming the first indication of the fasciculus longitudinalis medialis. By the close of the twelfth day, their axones have reached the anterior border of the anlage of the pons. They seem to grow but little farther during the next two days.

During the latter part of the twelfth and the thirteenth day, several commissural cells differentiate out of the floor plate in the pons. These cells resemble the floor-plate cells in Amblystoma (Coghill, '24). The axones of the above-mentioned portion of the fasciculus longitudinalis medialis seem to end among these commissural cells. In turn, the axones of these floor-plate cells seem to be continued in the fasciculus longitudinalis medialis of the opposite side.

Caudally to the pons, the fasciculus longitudinalis medialis is continuous with the fasciculus proprius of the ventral funiculus of the spinal cord and is formed during the thirteenth day of gestation. The greater number of neuroblasts forming this part of the fasciculus longitudinalis medialis arise between the motor neuroblasts and the floor plate; others lie farther dorsad. Most of these cells are commissural in nature.

42 a. *Studies on the facial musculature of the monotremes compared with the marsupials and placentals.* ERNST HUBER, Department of Anatomy, Johns Hopkins University.

The arrangement of the facial musculature in the monotremes can be derived from a common ground-plan which differs fundamentally from the marsupio-placentalian plan.

Common to both Echidna and Ornithorhynchus is a heavy longitudinal muscle mass ('platysma') which extends backward into the domain of the panniculus carnosus, interlacing and partly fusing with this muscle. Powerful portions of the 'platysma' insert into the fore limb, while the rest passes below the ear into the face, where it becomes firmly attached to the base of the rigid snout. Facial muscles are grouped around ear and eyes.

In both monotremes there is a powerful sphincter colli, which in Ornithorhynchus lies throughout its entire extension external to the 'platysma,' while in Echidna a small part, caudal to the ear, dips under it. In both, the external sphincter extends forward as far as the base of the snout.

The absence of a continuous sphincter colli profundus is significant. The author considers the m. bursae buccalis of Ornithorhynchus and 'm. buccinator' of Echidna as deviations from the longitudinal layer, and not as derivatives of a sphincter colli profundus. Notable differences in the snout musculature of the two monotremes may be explained as divergent specializations in connection with the elongation of the snout in Echidna and the formation of a bill and acquisition of a buccal pouch in Ornithorhynchus.

42 b. The cutaneous field of the n. trigeminus in the monotremes compared with the marsupials and placentals. ERNST HUBER, Department of Anatomy, Johns Hopkins University.

The cutaneous branches of the trigeminus in the monotremes show an arrangement which differs fundamentally from that of the marsupials and placentals. Emerging through a series of definitely placed foramina in the nasal, maxillary, at the junction of the maxillary with the premaxillary, and at various points on the inner and outer surfaces of the mandible, these trigeminal branches concentrate on the naked snout, which thus possesses plentiful innervation. The trigeminal branches are unusually big in Ornithorhynchus, which is largely guided by the tactile sense of the snout. In Echidna, in which the olfactory sense greatly dominates the tactile sense of the snout, the cutaneous trigeminal branches are accordingly smaller.

The arrangement of the cutaneous trigeminal branches in the marsupio-placentalian ground-plan, on the other hand, shows definite relations to the facial tactile vibrissae.

Considering the peculiar arrangement of the trigeminal branches and the occurrence of special tactile structures connected with the terminal twigs of these sensory branches in the monotremes, and giving weight to the consideration that the monotremes in their plan of facial musculature show no traces of muscles comparable to those which in the marsupials and placentals have differentiated as facial-vibrissae movers, the author concludes that the monotremes have, in contrast to the rest of the mammals, never possessed facial tactile vibrissae.

42 c. Conclusions as to the genetic relationship of the three phyla of the mammals. ERNST HUBER, Department of Anatomy, Johns Hopkins University.

The sriking peculiarities which the monotremes exhibit in their ground-plan of facial musculature and cutaneous field of the trigeminus, together with a whole series of further characteristics by which the monotremes fundamentally differ from the rest of the mammals, lead to the assumption that the monotremes must have derived fully apart from the marsupials and placentals, from a different group of the theriodont reptile stock. The marsupials and placentals, on the other hand, show such striking resemblance in their ground-plan of facial musculature, in the restriction of the tactile surface of the snout to the rhinarium, in the possession of facial tactile vibrissae, in the distribution of cutaneous branches of the trigeminal, and in the arrangement of the foramina through which these branches emerge from the skull, that these characteristics cannot possibly be explained as mere coincidences, but must surely be considered as the result of common ancestry.

43. The developmental potencies exhibited by the modified ('freemartin') ovary of Amblystoma tigrinum following removal of the testis which had induced this modification. R. R. HUMPHREY, University of Buffalo.

By removing the preprimordium of one gonad at stages 24 to 32 and replacing it with the corresponding preprimordium from another embryo of the same age, an ovary and a testis may be caused to develop concomitantly in the same animal. Under these conditions the testis usually develops normally, while the ovary undergoes modifications which reduce it to a rudimentary or vestigial state. In this condition the increased prominence of its hilar and medullary portions and the degenerate condition of its cortex give it the appearance of a testis rather than an ovary. Upon removal of the testis which has induced this modification, however, the gonad quickly regenerates as an ovary. The oogonia remaining in its cortex proliferate, oocytes begin their growth period, and the ovarian cavity is reestablished.

If, previous to removal of the normal testis, the modified ovary has begun development in some portion of its extent as a testis, this region may inhibit the regeneration of the ovarian cortex, permitting the gonad to continue its transformation into a testis. Thus, although a freemartin ovary commonly regenerates as an ovary, it may complete its reversal into a testis if this process is sufficiently advanced at the time of removal of the testis which induced it.

44. On several characteristics of the spinal nerves in the albino rat, according to sex. K. IDE (introduced by Henry H. Donaldson), The Wistar Institute of Anatomy and Biology.

At the Rochester meeting observations were reported which showed that, in the albino rat, the sectional areas of the median and sciatic nerves were respectively greater in the female than in the male of like body length. It has since been found that the sectional area of the largest fibers in these nerves is similarly larger in the female.

On examining the dorsal and ventral roots of the lumbar nerves, there were found in each root the same relations that had been found in the sciatic nerve, both for the total area and area of the fibers.

The conditions observed in the sciatic nerve are, therefore, found in the constituent nerve roots also. Study of the data shows that when these areas are alike in the two sexes the corresponding body sizes are those to be expected at like ages, the female being a smaller animal than the male at the same age.

If inference may be made from the nerves examined to the entire system of spinal nerves, then it can be concluded that this system grows in the same way and to the same extent in both sexes, but, as the body growth in the female is less than in the male, the sectional areas are larger in the female when the sexes are compared at the same body length.

45. Observations on functional changes in sympathetic-ganglion cells in the albino rat. H. E. INGERSOLL (introduced by A. Kuntz), Medical College of Virginia.

The sympathetic-ganglion cells of the coeliac plexus of the albino rat were studied for evidences of change due to stimulation of the abdominal viscera. The cells were stained with erythrosin-toluidin after mercuric chloride-formalin fixation. The ganglion cells were classified according to the density, appearance, and distribution of the extranuclear chromatic substance (Nissl bodies), together with changes in the nucleus.

The principal types of cells encountered were as follows: cells with an even distribution of extranuclear chromatic substance which apparently were normal resting cells; intensely staining cells; cells with the Nissl substance collected around the nucleus; others with the chromatin around the periphery of the cell; and a group of very pale cells with considerable evidence of exhaustion.

The largest number of resting cells was found in the controls, with a rapidly decreasing percentage as stimulation increased. The densely staining cells increased in number in the early stages of stimulation, but decreased as stimulation progressed. The perecentage of pale cells varied with the degree of stimulation, the smallest number being found in the controls.

46. *Further observations on the structure of the myenteric plexus.* SYDNEY E. JOHNSON AND MARTIN PALMER, School of Medicine, University of Louisville.

Recent publications of Lawrentjew, Trostanetzky, and others have brought up once more the question of the presence in the gut wall of a primitive nerve mechanism of syncytial structure. The interstitial cells are claimed by different authors to be nerve cells, glia cells, or some form of connective-tissue cells. In a study of the cells of the myenteric plexus with various connective-tissue (Mallory's acid fuchsin, aniline blue, etc.) stains, several neuroglia stains, as well as neurological methods, we have found only connective tissue and the well-known large ganglion cells and their processes. Special neuroglia stains have so far yielded negative results.

47. *The effect of injections of female sex hormone on conception and pregnancy in the guinea-pig.* G. L. KELLY, Department of Anatomy, University of Georgia.

Injected immediately after copulation in adequate dosage, female sex hormone will always prevent conception in this animal. Dosage averaged about 90 rat units, given in six equal injections on successive days. Smallest effective dose—15 rat units in three 5-unit injections.

Two weeks' pregnancy was terminated by abortion in seven out of eight cases, using from 75 to 150 rat units.

Among animals four weeks pregnant, thirteen abortions occurred in nineteen cases; five animals died with retained fetuses; one delivered about term. Average dosage, 150 to 300 rat units; minimum, 90 units; maximum, 700.

The retained fetuses were macerated and the uteroplacental junction was necrotic.

Twelve animals six to eight weeks pregnant received from 600 to 1150 rat units. Two miscarried and lived; ten died with retained fetuses. Five others received from 300 to 400 units and all died with retained fetuses.

Non-pregnant females and males injected with large doses of female sex hormone proved that the deaths of the animals of more advanced pregnancy were not due to toxicity of the injections. An explanation of all these deaths is offered.

Animals that received 1 per cent peptone injections invariably went on to delivery at term and only one that received them after copulation failed to conceive.

A microscopic study of the ovaries, uterus, and placenta was made.

48. The primary lymphatic system of frog embryos as seen in injections. HENRY McE. KNOWER, The Wistar Institute of Anatomy and Biology.

A brief discussion of some important aspects of this system of young embryos of Rana palustris and Rana catesbiana studied in injections. Selections from a monograph in preparation.

Early lymphatics are here correlated with stages of veins and organs to secure more accuracy in comparative studies. Special attention is devoted to the earliest stages of the anterior lymph hearts in relation to their plexuses and to connections with a supraneural plexus from which the 'dorsal lymphatic' arises. This trunk is not developed in these forms from separate rudiments. It is in continuous connection with the anterior lymph hearts and their plexuses from their first appearance. The capillaries of lymphatic plexuses connect with venous capillaries in early stages.

The history of the extension (plexuses), from which the temporal, maxillary, and other cephalic sacs (sinuses) arise, is followed in injections from a stage immediately after the appearance of the anterior lymph hearts. The parts of this cephalic system form a continuous plexus draining into the anterior lymph hearts from the start through the 'jugular lymphatic' ducts. The cephalic sacs (sinuses) are therefore not formed in these embryos from separate rudiments. A comparison with similar vessels in Amblystoma and Necturus embryos reveals a homologous system, no part of which, however, becomes distended into sacs in these urodeles. The histories of the lateral and ventral lymphatics are also followed.

49. Development of vagina in the human fetus. ARTHUR K. KOFF (introduced by G. L. Streeter), Department of Embryology, Carnegie Institution of Washington, Baltimore.

Tubo-uterine junction lies at caudal end of mesonephros where müllerian ducts bend medially to cross wolffian ducts. The boundaries between cervix, vagina, and body of uterus are seen already in 25-mm. embryos, at which time cervix consists of spindle-shaped thickening of mesoderm. The cervicovaginal junction becomes definitely marked between 80 and 90 mm. by differentiation of surrounding mesodermal condensation, which is before appearance of solid primordia of fornices.

The epithelium of upper two-thirds of vagina is derived from müllerian ducts; the lower one-third appears to be formed by coalescence of lower ends of wolffian ducts and upper portion of urogenital sinus.

Four stages can be defined in development of vagina: 1) 25 to 60 mm., before coalescence of wolffian ducts with sinus; 2) 60 to 90 mm., wolffian coalescence and transformation of upper two-thirds of vaginal canal into a solid cord; 3) 90 to 126 mm., proliferation of solid vaginal primordium from one central and two lateral foci; 4) degeneration of central cells with resultant vaginal canal.

In embryos up to 40 mm. growth in length of uterovaginal canal is mainly in cervical portion. In specimens 40 to 125 mm. growth is mainly in region of future vagina.

50. Hermaphroditism and pseudohermaphroditism in Homo; a comparison. S. I. KORNHAUSER, School of Medicine, University of Louisville.

Two children, one died ten days after birth, other three weeks. One, a true hermaphrodite, previously described—two testes and epididymides, two ovaries, two uterine tubes, uterus, one ductus deferens. Ovaries of true hermaphrodite contained well-developed graafian follicles, but of relatively small size with small antra. Largest follicles measured 0.42 × 0.35 × 0.24 mm. and 0.39 × 0.34 × 0.21 mm. The pseudohermaphroditic child showed external genitalia of intermediate type from which sex could not be determined, large penis-like clitoris, labia majora scrotum-like, but no testes palpable. Labia united and continuing as ridges on the underside of clitoris. Internally female. No testes found after complete search. Sections made of all organs. Gonads and mesonephric structures cut completely and serially. The following peculiarities were noted: large epoophora on both sides, cuboidal germinal epithelium had become squamous except in a few places and no proliferation seen even where it did remain, tunica albuginea considerably thickened, much beyond normal. Formation irregular cellular cords in the cortex beneath tunica containing some germ cells. These cords not very similar to seminiferous tubules. Well-developed graafian follicles with large antra. Four largest follicles measured 4.28 × 2.91 × 2.47 mm., 2.47 × 1.42 × 0.78 mm., 1.98 × 1.31 × 0.84 mm., 1.72 × 1.50 × 0.71 mm. Most interesting fact that, in spite of great differences in gonads of the two individuals, development of external genitalia very similar. In true hermaphrodite presence of testes did not greatly suppress development of ovaries.

51. On the occurrence of myelinated internal carotid nerve fibers which pass through the superior cervical sympathetic ganglion. ALBERT KUNTZ, St. Louis University School of Medicine.

Osmic-acid preparations of the internal carotid nerve in the cat reveal the existence in this nerve of some myelinated fibers of larger caliber than the usual postganglionic sympathetic fibers. These fibers undergo myelin degeneration following section of the cervical sympathetic trunk between the middle and superior cervical sympathetic ganglia. It must be assumed, therefore, that they pass through the latter ganglion.

52. The cerebellum of Triturus torosus. O. LARSELL, University of Oregon Medical School.

Triturus shows the histological transition from the relatively simple structure of the bulb to the differentiated pattern of the cerebellum. Auricular lobes are prominent, as is also the acousticolateral area. The corpora cerebelli are joined by a thick plate. The fibrous layer of the acousticolateral area is continuous around the auricular lobe with the molecular layer of the cerebellum. Very fine unmyelinated fibers, present in the acousticolateral area as branches of lateral-line fibers, continue into the molecular layer. This fibrous area is interpreted as being homologous in part to the cerebellar crest of fishes. The cerebellar commissure connects the anterior trigeminal regions of the two sides and may be regarded as an intertrigeminal commissure, in a sense. The cells which give rise to its fibers lie in the ventral part of the corpus cerebelli, and send dendrites into the trigeminal areas. Their axones constitute the commissure. The unmyelinated lateral commissure arises from small cells of the auricular lobes and may be regarded as an interauricular commissure connecting static areas. These cells and fibers are closely related to granule cells and their fibers of higher forms. The VIII tract reaches the auricle primarily, and some of its fibers decussate with the interauricular commissure. A trigeminocerebellar tract reaches the cerebellar region. Other fiber tracts are similar, in general, to those in other urodeles.

53. *The effect of x-ray upon the offspring of albino rats.* GEORGE LEIBOLD AND T. T. JOB, Loyola University School of Medicine.

Apparently the size of the dose of x-ray determines the injury done to the embryo or fetus. Eight-tenths skin unit had its maximum effect on fetuses irradiated between the seventh and ninth days of gestation, complete resorption resulting. While resorption was possible in animals irradiated before the seventh and after the ninth day of gestation, viable litters were delivered. Living young showed no abnormalities at birth. Six instances of a skull defect have appeared by twenty days, and, on an average of forty days, several instances have been observed where there were a bleeding nasal mucosa, inflamed eyes with an exudate, shaggy fur, underweight, inactive and anemic condition. This suggests the need of reserve in interpreting effects of x-ray, administered during gestation, on the young at birth. The ovaries are affected in this treatment also, since some of the young in succeeding litters showed similar defects to those noted in the first litter after irradiation. In one instance, two young of the sixth litter after treatment exhibited skull deformities.

54. *Mathematical precision in the apparently fortuitous areas of cells in epithelial mosaics.* FREDERIC T. LEWIS, Harvard Medical School.

In the actively growing epidermal epithelium of a 100-mm. cucumber, the average area of pentagonal cells was found to be 25 per cent less than the average area of the hexagons, and the area of the heptagons 25 per cent greater. Thus the area of the hexagons is a mean from which the areas of pentagons and heptagons differ by 25 per cent. In the less active epithelium of a full-grown cucumber this deviation, on the basis of 300 cells carefully measured, is reduced to 22 per cent. More strikingly the equalizing tendency is shown in the amnion of a retained human ovum (24-mm. embryo). Studied in surface view, 100 hexagons, heptagons, and pentagons were measured, ranging from 55 to 231 sq.μ. Average area of the 300 cells, 117 sq.μ; 100 hexagons, 116 sq.μ; 100 pentagons, 101 sq.μ; 100 heptagons, 134 sq.μ. The hexagons continue to be intermediate between pentagons and heptagons, which here differ from them by only 14 per cent. Coincident with this equalization, certain cells with pycnotic nuclei have become small four-sided or three-sided forms, essentially eliminated from the epithelium. A comparable readjustment with three-sided eliminations will be shown in photographs of a layer of soap bubbles. In the amnion, therefore, though pentagons and heptagons differ equally in area from the hexagons, the difference is much less than in the active epithelium of the cucumber.

55. *Moving pictures of normal cells.* Warren H. Lewis, Department of Embryology, Carnegie Institution of Washington, Baltimore.

Each cell type has its peculiar mode of migration. Lymphocyte migration is intermittent. From rest and spherical form they suddenly dart off, elongate, and move for one to several minutes, then stop, become spherical and rest for one to three minutes. The average rate of progression is 0.019 mm.; the most rapid, 0.0348 mm. per minute. The elongated migrating lymphocyte has an anterior end with pseudopodia, an oval body, and a tail. The anterior end usually persists during a migratory period and is the only point of attachment. Lymphocytes tend to move zigzag in a given direction, but may sometimes turn about. Polymorphonuclear migration is continuous. They dart about in various directions. The anterior end tends to shift from one part of the cell to another, more frequently than with the lymphocytes. Contraction waves pass from the anterior to the posterior end and often produce a blunt tail. Macrophages rapidly change form and move about in an irregular manner by means of large thin waving film-like pseudopodia which vary enormously in extent under different conditions. By means of these pseudopodia they take up débris and fluid globules of various sizes. The latter pass from the periphery to the body of the cell, often fuse on the way, and then rapidly shrink. Under certain conditions, relatively enormous amounts of fluid thus pass through these cells.

56. *Motion picture of the rabbit's colon, showing independent action of the three rows of taeniae.* P. Lineback, Emory Medical School.

The movements of the colon have been studied by Biederman, Contrad and Guyon, Kaestle, Katsch, and Thaysen. Alvarez, more recently, has done some valuable work upon the rabbit's colon and has registered the working of the bowel on motion films.

The author has studied the movement of the unstimulated saccules in the rabbit's colon by motion film and notes that they may, and commonly do, act independently.

57. *A study of the reticulum (argyrophile fibers) in the ovary of the albino rat.* J. B. Looper and Margaret Haase Looper, University of Mississippi School of Medicine.

The material includes ovaries taken from rats at different periods of pregnancy, and from virgin rats which vary from one day to eight months in age. In the preparation of the material Foot's reticulum technique was employed. As controls the following stains were used on different sections of each specimen: anilin blue collagen, van Gieson's picro-acid fuchsin, and phosphomolybdic acid-hematoxylin. Argyrophile fibers in abundance are distributed among the other fibrous elements of the tunica albuginea, trabeculae, blood vessels, and theca folliculi. Likewise, such fibers occur in the cortex and medulla of the ovary, but here they form a more or less narrow-meshed stroma. The membrane propria of the theca folliculus essentially is a compact membrane composed of delicate argyrophile fibers. Similar fibers continuous with this membrane form the early stroma of the corpus luteum. The argyrophile fibers gradually decrease in abundance as the corpus luteum is transformed into the corpus albicans. This is interpreted to be in accord with the view that such fibers are precursors of true collagenous fibers.

In the atretic graafian follicle the membrane propria is greatly hypertrophied. Despite this fact, the membrane retains its argyrophilic properties. Thus it is indicated that such properties are not dependent upon the size of the fibers, and that argyrophile fibers are not identical with finely divided collagenous fibers.

58. *The structure and activity of the ciliated epithelium lining the vertebrate fallopian tube.* Alfred M. Lucas (introduced by E. V. Cowdry), Washington University Dental School, St. Louis.

Parker's ('28) observation that abovarian and proovarian ciliary tracts occur in the fallopian tube of the turtle has been confirmed in the present work upon this animal.

The epithelium of the fallopian tube of the guinea-pig has been studied with the tube in situ and after its removal from the animal. Ciliated cells are most numerous at the fimbriated end and decrease in number toward the uterus. Except at the fimbriated end, they occur either singly or in small groups among the non-ciliated cells. The cilia average 2.3 μ in length.

The cilia of the human fallopian tube are longer, averaging 4.3 μ. Ciliated cells are numerous throughout the length of the tube and are arranged din the epithelium to form a network of anastomosing tracts. In material observed thus far the direction of current has always been toward the uterus, though with additional material taken at all periods of the menstrual cycle the proovarian tract may be found. Particles are moved by the cilia at a rate varying from 4 to 50 μ per second. The distance which lies between the particle and the epithelium is largely responsible for this variation. Particles distant 84 μ from the epithelium have been influenced by ciliary activity. Great variations in form and size of ciliated and non-ciliated cells have been noted.

I am indebted to Dr. Otto Schwartz, of St. Louis Maternity Hospital, for this opportunity to study living human tissues.

59. *Comparison of effects of temperature variation with high-frequency oscillations.* Jean MacCreight and G. Murray McKinley (introduced by H. H. Collins), University of Pittsburgh.

Week-old albino rats exposed to an electrostatic field obtained by an apparatus giving a frequency of 100,000,000 cycles per second were killed in three minutes. All exposures were made in an open glass tube at an auxiliary current of 3 amperes. Saline-thermometer readings of this output did not reach blood temperature in the time required for lethal dosages. Death of exposed animals was preceded by a severe rush of blood to fore and hind limbs and tail, these appendages becoming severely congested and swollen. To determine what degree of pure heat would be necessary to parallel the rush of blood and subsequent congestion induced by high frequencies, rats of the same age were subjected to the following degrees of heat: 55°, 66°, 80°, 145°, and 160°C. It was only when the temperature reached 160° that the effects were fairly comparable to those obtained by high frequencies. Abdominal temperatures taken were on the average the same as for high frequency, the highest being 45°C. Body regulation is able to keep the temperature below 46°C. whether the exposure be in an electrostatic field or in pure heat. Neither rectal or saline thermometers are measures of the actual heat to which an animal may be subjected when exposed to high frequencies.

60. *Respiratory movements in the smaller bronchi and bronchioli as shown by stereoscopic x-ray films with the aid of injected lipiodol.* CHARLES C. MACKLIN AND PAUL M. ANDRUS, Department of Anatomy of the University of Western Ontario and Queen Alexandra Sanatorium, London, Ontario.

Lipiodol, when injected into the bronchial tree, throws a shadow on x-ray films which gives information as to the shape of the part which is filled. If used in moderate quantity and allowed to spread evenly over the bronchial wall, the tubes still transmit air readily.

Restricted areas of the bronchial tree were injected thus in healthy human subjects, and stereoscopic x-ray films taken at the end of full expiration and full inspiration, with the patient standing in exactly the same position before the casette. The films were split, and corresponding halves of expiratory and inspiratory phases were mounted side by side and studied in a stereoscopic x-ray view-box. An easy means of comparing the structural changes in the bronchi in inflation and deflation of the part was thus afforded, for stereoscopic views of the same part of the bronchial tree in the contrasted conditions of expiration and of inspiration appeared in the same optical field.

An elongation with widening of the tubes was very evident in inspiration, and a shortening with narrowing in expiration. No material alteration of the interbronchial angles was noted. These results are in keeping with those previously obtained for the larger tubes by one of us (C. C. M.).

61. *Experimental alteration of hepatic-cell protoplasm by exposure to circulating blood diluted with water.* MADGE THURLOW MACKLIN AND CHARLES C. MACKLIN, Department of Anatomy, University of Western Ontario.

The water content of the blood flowing through the liver sinusoids may be increased in a number of ways, one of the most effective being the injection of water into the peritoneal cavity. The water is quickly absorbed by the tributaries of the portal vein, and dilutes the portal blood.

A number of cats and other animals were so treated, and the livers examined at various intervals thereafter. In a cat, for instance, at the end of only one and one-quarter hours after the injection of 100 cc. of distilled water, all the hepatic epithelial cells were full of vacuoles, and were much swollen, the sinusoids being greatly narrowed and even collapsed. The protoplasm of the cells looked like froth. All animals so treated showed this change. It persisted, after a single injection, for many days, particularly in the portal zone. Introduction of a similar quantity of distilled water into the small intestine produced the same effect on the liver cells, but in lesser degree, and mainly in the portal area. Similar results followed perfusion of the liver with distilled water.

These findings have a direct relation to the mechanism of protection of the liver from too great dilution of the portal blood with water from the intestinal tract.

62. The physiological activity of the hypophyses of rats under various experimental conditions. K. E. Mason and J. M. Wolfe, Department of Anatomy, Vanderbilt University School of Medicine.

Transplantation of hypophyses of castrated rats induces a greater response in the genital tract of immature female rats than do those of normal rats (Engle, '29; Evans and Simpson, '29). This same method is being used to test the physiological activity of the hypophysis in rats subjected to various dietary deficiencies, as one phase of a study of vitamin and hormone interrelationships. Young rats, about twenty-five days of age, were placed on an A-deficient diet. One-half of these were castrated. When xerophthalmia became well advanced, the hypophyses of control and castrate brothers, together with the hypophysis of a normal of the same weight and sex, were transplanted subcutaneously into immature litter-mate females. The ovaries of the test animals receiving hypophyses from the A-deficient non-castrates averaged 43 per cent greater than those receiving hypophyses from normal stock controls. The hypophyses of A-deficient castrates induced a still greater reaction in the immature rat ovaries—averaging 39 per cent greater than the A-deficient non-castrates and 100 per cent greater than the stock controls. The hypophyses of male rats subjected to chronic inanition showed a significant decrease in activity when contrasted with hypophyses of, 1) litter-mate brothers fed unlimited amounts of the same diet; 2) stock controls of the same body weight. Similar tests are being carried out on rats subjected to B and E deficiency.

63. Dermatoglyphics in primates, with particular reference to man. Charles Midlo (introduced by A. H. Schultz), Laboratory of Physical Anthropology, Johns Hopkins University.

Dermatoglyphic evidence indicates that the distal (interdigital) parts of the palm and sole of primates are subjected to more diverse functional influences than are the proximal (thenar, hypothenar, calcar) areas.

Old World monkeys show, in correlation with hypertrophy of volar pads, expansion of patterns and marked retention of the primitive pattern type (whorls). In general, New World monkeys have least deviated from the fundamental plan, demonstrating many primitive conditions in ridge alignment, placement of triradii, and pattern forms. In similar respects and outweighing by far the concurrent specializations of certain dermatoglyphic features, the palmar and plantar dermatoglyphics in man are found to be considerably more conformant to the fundamental dermatoglyphic plan than are those in gibbon and in anthropoids. The latter manifest greater distortion of dermatoglyphics and marked failure of the first plantar interdigital pattern which occurs in 96 per cent of human soles.

In sample series of platyrrhines (marmoset: 26 palms, 26 soles; squirrel monkey: 19 palms, 20 soles), lower catarrhines (baboon: 26 palms, 58 soles; macaque: 71 palms, 118 soles), man (about 2000 palms and as many soles), and anthropoids (orang: 17 palms, 19 soles; chimpanzee: 17 palms, 25 soles; gorilla: 6 palms, 11 soles) the degree of collective dermatoglyphic variability increases in the order listed. The relative stability of dermatoglyphic features found in gibbon (38 palms, 111 soles) is explainable on the basis of extreme specialization.

64. *The reaction of the rat vas deferens to castration and to testis-hormone injection.* CARL R. MOORE, S. VATNA, AND T. F. GALLAGHER, The University of Chicago.

The rat vas deferens exhibits marked degeneration changes after testis removal. Twenty days after castration, the normal ciliated border of the epithelial lining is almost or entirely absent and the epithelium exhibits shrinkage of the cells, nuclear stratification, and modified protoplasmic changes. Within a few weeks the musculature is involved and the entire duct is approximately one-half its normal diameter. The castration changes are sufficiently well marked and develop with sufficient rapidity after operation that the condition of the tube can well be utilized as a testis-hormone indicator.

Subcutaneous injections into castrated rats of lipoid extracts of fresh bull testes have shown that the preparations injected contained the testis hormone.

By injections of potent preparations into castrated males we have been able, 1) to maintain the normal conditions for the vas deferens indefinitely; 2) to restore to a normal condition the vas deferens of adult rats castrated for many months; and, 3) to bring to a normal condition the vas deferens of rats that were castrated before attaining puberty and that had lived for months after castration. Normal return is obtained within twenty to thirty days of injection despite a degenerate condition of several months' standing.

Injections of the testis hormone must be continuous (daily) to be effective, since the hormone is excreted by the kidneys.

65. *The rôle of the tuber cinereum and the thyroid gland in experimental fever in the dog.* LAWRENCE O. MORGAN, University of Cincinnati College of Medicine.

The author has previously described, following irritative lesions in the tuber cinereum, convulsions which resembled those of human epilepsy. The convulsions usually increased gradually in severity and frequency, terminating in a condition resembling status epilepticus. The temperature rose with the increase in frequency of the spasms, usually reaching 109° to 111°F. Ligating the blood supply of the suprarenals caused a cessation of the 'autonomic' symptoms (dilatation of pupils, salivation, vasoconstriction, increase in heart rate and blood pressure, etc.). Ligating the thyroid glands caused the muscular spasms to cease and the temperature to fall.

These experiments suggested that certain types of fever may be concerned, to a large degree at least, with a hyperactivity of the thyroids due to the action of a toxin upon a secretory center in the tuber cinereum. Experimental fever was produced in eleven dogs over a period of one to three days by repeated injections of either typhoid or bronchioseptus toxin. In these animals ligation of the vessels of the thyroid was followed by a rapid drop in the temperature.

A microscopic examination was made of cell groups in the midbrain, diencephalon, striatum, and cortex. The nucleus tubero-mamillaris in the tuber cinereum was the only cell group which was found to show significant changes. Thirty to fifty-five per cent of the cells in this nucleus were definitely chromatolytic. Many other cells showed a marked reduction in the Nissl granules.

66. *Movements in transplanted limbs innervated by eye-muscle nerves.* J. S. NICHOLAS, Osborn Zoölogical Laboratory, Yale University.

The limb bud of Amblystoma punctatum was substituted for the developing optic cup to test the growth of the nerves and the function of such limbs. All animals in which fusion occurred between the cartilage from the transplant and the developing skull were discarded, since the physiological correlation of the limb movements with movements of the eye of the opposite side was quite limited.

Four animals were obtained in which the transplanted limb was innervated by eye-muscle nerves and one in which the motor innervation was secured through the motor component of the trigeminal. These were studied physiologically and later these results were controlled by histological examination.

The greatest range of functional movements was obtained in one case which was innervated through the oculomotor nerve. This limb performed movements coincident with movements of the eye of the unoperated side. The limb was extended and flexed, slightly abducted and adducted; there was slight flexion and extension of the forearm upon the arm. In the limbs innervated by the abducens and the trochlear, movements were exceedingly limited, the abducens producing abduction, the trochlear adduction. The limb innervated by the motor branch of the trigeminal showed strong flexion and weak extension.

67. *A hitherto undescribed insertion of the temporal muscle.* GUSTAVE J. NOBACK, Department of Anatomy, New York University.

Examination of a series of 130 human cadavers shows the presence of an insertion of the temporal muscle which has not been recorded in the literature.

A fleshy portion of the temporal muscle leaves its medial aspect, passes medially, inferiorly, and anteriorly to the posterior part of the lateral surface of the buccinator muscle, along which it is attached for some distance.

This muscle band was present on both sides in all cases, with the exception of two old females, in which it was definitely present on one side and represented on the other by a fibrous band.

This portion of the temporal muscle ranges from a large, long, fleshy belly to one which arises from the temporal as a fleshy belly, but which soon becomes tendinous.

In most cases the buccinator nerve and vessels pass through a space bounded medially by the muscle band and laterally by the temporal muscle on the medial aspect of the coronoid process, and in some, it was observed that branches of the buccinator nerve passed into the substance of the muscular band.

68. *Diurnal variations of height and weight in the human body during growth.* CARROLL E. PALMER, Institute of Anatomy, University of Minnesota, and Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University.

Measurements of height and weight were obtained upon three children at approximately hourly intervals during the day for from three to five consecutive days. The results were combined with the available published data on daily fluctuations of these measurements.

During the first hour after arising, height decreases approximately 7 mm.; during the second, approximately 3.5 mm.; during the third, 2.5 mm.—with a total decrease of 15 mm. in four hours. Thereafter reduction is less, cumulating in a total of 25 mm. at the end of sixteen hours. Variations are essentially the same regardless of the age of the individual and represent, therefore, the equivalent of the growth increment of intervals of from two to twelve months. Diurnal variations in weight are influenced by artificial factors which make impossible a general statement of changes during the whole growth period. Usually the weight of infants does not increase more than 300 grams during the day; the weight of preschool children (two to five years), not more than 500 grams; and of older children, not more than 1000 grams. These fluctuations are equivalent to growth increments of from two to six months. Variations of height and weight are considered in connection with the reliability of these measurements and in connection with the evaluation of cyclic manifestations of growth.

69. *The possible relations of the development of the suprarenal gland to the origin of foetal movements in the albino rat.* D. S. PANKRATZ (introduced by G. E. Coghill), University of Tennessee.

The suprarenal gland in the albino rat appears first in the thirteen-day-old foetus. A slight thickening of the coelomic epithelium medial to the cephalic portion of the mesonephros marks the anlage of the suprarenal cortex. Growth at the time is rapid, and during the fifteenth to the sixteenth days the cortex forms an oval mass protruding into the dorsal coelomic cavity. About the sixteenth day, just before foetal movements begin, cells migrate from sympathetic ganglia toward the cortex. At the time (sixteen days or more) when foetal movements have been first observed by Swenson and by Angulo, a definite mass of sympathochromaffin cells is located among the medial cell cords of each cortex. This cell migration continues during the seventeenth and eighteenth days and possibly till birth. In the seventeen-, eighteen-, and nineteen-day foetuses nerve fibers which connect the gland with the sympathetic chain are observed with large ganglionic cells along their course.

From the literature on migration of the sympathochromaffin cells, on first chromaffin reaction and appearance of foetal movements in the chick, mouse, and man, the author concludes that in these a correlation similar to that in the rat might exist. Possibly this initial activity of the suprarenal medulla causes an increase in the irritability of the muscular or neuromuscular system, and thus facilitates the beginning of foetal movements.

70. *Certain measurements of the foetal heart and their significance.* BRADLEY M. PATTEN AND KATHRYN TOULMIN, Laboratory of Histology and Embryology, School of Medicine, Western Reserve University.

Preliminary report on average normal conditions in the human heart at the time of birth. Material assembled with a view to furnishing a basis of comparison for evaluating abnormalities of the neonatal cardiovascular system. Data include total heart weight, total ventricular weight, relative weights of right and left ventricles, and cross-sectional areas of main vascular entrances and exits. Discussion of the functional implications of these measurements with reference to foetal circulation and changes in circulation following birth.

71. *Acoustic insulation and the efficiency of the sound-transmission system.* A. G. Pohlman, St. Louis University School of Medicine.

The development of the internal ear assures an acoustic insulation because it is an immersion receptor. This insulation is overcome by the sound-transmission apparatus which compensates for the impedance differences between air and water. The insulation efficiency may therefore be computed on the basis of the lowered air acuity in cases where the sound-transmission apparatus is out of commission and this may be represented by a factor of 3000 to 10,000 times the normal threshold intensity. The evidence shows that the leverage action of the ossicular chain is not related to sound transmission and that the round window is not essential to pitch analysis or to the sensitivity of the end-organ response. The morphology of both the middle and the internal ear must therefore be interpreted not only in relation to the function of sound transmission and of sound analysis, but also from the standpoint of a shock-absorber system.

72. *Radiological investigation of the time element in ossification.* J. W. Pryor, University of Kentucky.

The conclusions of the author are based on the examination of 1024 roentgenograms. Of this number, there are 140 foetuses from ten and one-half weeks to thirty-eight weeks of age; one hundred babes from one hour to seven days old; 554 children from three months to twelve years old, and 300 from twelve to twenty-four years of age.

In this investigation the time of appearance of centers of ossification in the bones of the carpus, tarsus, the epiphyses of the metacarpals and phalanges of the hand; the distal epiphysis of the radius, ulna, and femur, and the proximal epiphysis of the tibia were noted in both the male and female and comparisons made.

A large number are brother and sister; there are six from one family and four in another family. This includes several sets of twins of the same age and of opposite sex. It is the opinion of the author that with certain groupings of these centers, noting the time of appearance, the size of the center in millimeters, and the absence of other centers, and, in the older instances, the time of union of the epiphyses with the diaphyses, the determination of sex could be made, by this method, in a large percentage of instances.

73. *Standing and stepping as exhibited by spinal cats.* S. W. Ranson and J. C. Hinsey, Northwestern University Medical School.

In cats the spinal cord was transected at levels varying from L 6 to T 4, and time up to three weeks allowed for recovery. Within a few days after the operation, the animals with sections at L 3 or above could stand, bearing the weight of the posterior half of the body on the hind legs for periods varying from twenty seconds to three minutes. The spinal portion of the reflex mechanism responsible for standing is complete below the level of the third lumbar segment.

The animals were also examined when supported in a frame with the legs hanging pendent. Stimulation of the skin of the abdomen and groin by the pressure of the body against the hammock reinforces the extensor tonus in the hind limbs when the animals are supported in this way. Under these conditions, upward pressure against the pads of the toes calls forth the support reaction described by Rademaker. It could sometimes be reinforced by pinching the tip of the tail and could always be inhibited by pinching the skin over the back of the sacrum.

Rhythmic stepping movements could be elicited by pinching the skin of the perineum and were best developed after the higher transections. After transections below the level of T 12, they could not be elicited or were poorly developed and difficult to obtain.

74 a. *The earliest stages in the development of omphalomesenteric arteries in the rat.* Franklin P. Reagan, Department of Anatomy, Medical School, University of Illinois.

Prior to the development of hindgut, the two dorsal aortae may terminate blindly, or, more developed, they meet to form a loop caudal to the prospective caudal end of the hindgut. In the latter condition the caudal loop gives a spray of arteries to the yolk sac. In the region of the prospective midgut, the dorsal aorta gives off small laterally coursing arteries reminiscent of early vitelline arteries of birds.

When hindgut forms, the caudal arterial loop gives off ventrally and cranially the subintestinal artery, which courses cranially to the level of the caudal intestinal portal. There it is continuous on each side with an arterial anastomosis located in the respective caudal velum of the midgut. Each anastomosis traced cranially is seen to join the respective transversely coursing vitelline artery at some distance lateral to its point of origin from the respective dorsal aorta. The part of vitelline artery medial to the point of union of vitelline artery and the artery of either arterial anastomosis with the caudal velum of the midgut may be called the basal portion of the vitelline artery. The remainder is its peripheral portion. The arterial anastomosis within each caudal velum now gives off the great caudal spray of arteries to the yolk sac.

The arterial anastomosis within the left caudal velum of the midgut and the basal portion of the left vitelline artery degenerate at stages slightly later than those covered by the present account. Subsequently, also, the umbilical artery arises from the ventrocaudal end of the caudal dorsal aortic loop and the base of the subintestinal artery.

74 b. *The earliest stages in the development of superior mesenteric arteries in the rat.* Franklin P. Reagan, Department of Anatomy, Medical School, University of Illinois.

Dense arterial capillary plexuses at the sides of the early hindgut connect the dorsal aortae with the subintestinal artery. Successively more cranially located diagonal enlargements take place in the dextral plexus, connecting the right dorsal aorta with the caudal end of the arterial anastomosis of the right caudal velum of the midgut. The basal portion of the dextral primitive vitelline vein degenerates. The peripheral portion of the latter is now supplied by the arterial anastomosis within the right caudal velum of the midgut.

The diagonally developed artery within the right arterial plexus of the hindgut now supplies the spray of arterial vessels off the arterial anastomosis within the right caudal velum of the midgut, and also the peripheral end of the right primitive vitelline artery. In the right plexus of the hindgut we have the beginnings of the migrating base of the superior mesenteric artery. As the left aorta fuses with the right, the vessel assumes the appearance of arising from a common dorsal aorta. Subintestinal artery has in the meantime degenerated.

This takes the history of a. mesenterica superior up to the stages investigated by Mall. A survey of the conditions in other mammals will supplement the description of conditions in the rat.

74 c. Thoracolumbar veins and dimorphic sex veins in embryonic and foetal pigs.

Franklin P. Reagan, Department of Anatomy, Medical School, University of Illinois.

By several different possible permutations and combinations, the three thoracic vessels constituting the true (non-posterior-cardinal) azygos system have 'normal' direct connection with the postrenal v. cava. These vessels course medially to the sympathetic trunks and medially to the peri-aortic sympathetic nervous plexuses. In addition to these normal azygos connections, the base of the subcostal vein on either side may be connected to postrenal v. cava by means of a persistent thoracolumbar vein. The dextral one most often persists. Where it does so, it joins postrenal v. cava directly. When the sinistral one persists, it joins the base of left renal vein. The doubled condition would seem to be more common than the persistence of the left thoracolumbar in the absence of the dextral one. The relative function of each affects profoundly the history of the true azygos lines. Thoracolumbar veins course laterally with reference to the nervous elements above discussed.

Primitive ovarian veins of the pig have a history similar to that of the female sex veins described by Reagan and Tribe for the rabbit. The male sex veins of the pig have a history varying from that given for the sex veins of any mammal.

75. The action of silver nitrate upon the myelinated nerve fibers of the frog (Rana pipiens).

George St. de Rényi, Department of Anatomy, Medical Department, University of Pennsylvania.

Living nerve fibers of the frog were exposed to silver-nitrate solutions of various strengths. All the changes in the fibers caused by this treatment were controlled with high-power lenses. It was noted that the action of silver nitrate depends primarily on the strength of this reagent. It was observed that the results of impregnating structures were different when the reaction was carried out on fibers possessing a connective-tissue sheath from those obtained with fibers from which this sheath had been previously removed. The true nature of the impregnated structure was revealed by the method of dissecting fibers treated with silver nitrate with the aid of the microdissecting instrument. It could be shown that the constricting band of Ranvier lies in the region of the connective-tissue sheath outside of the neurilemma and has no relation to the axon itself. By injecting silver solutions into the nerve fibers, the internodes could be directly exposed to the effect of the reagent. These experiments demonstrated that the indifference of the internodes to the silver nitrate as shown by Ranvier's original method cannot be explained on any other grounds except that the internodes differ from the nodes in their physical and probably chemical nature. Further injections gave no evidence that cement substance exists between the neurilemma segments.

76. The study of the lymphatic system of the thyroid gland in dog and in man. William Francis Rienhoff, Jr., Carnegie Laboratory of Embryology and the Surgical Hunterian Laboratory, Johns Hopkins University.

The lymphatic system of the thyroid of the dog was studied by injecting the lymph vessels with 10 per cent India ink at a distance from the gland, thus insuring an injection of only the lymphatic channels. The blood vessels were injected with a modification of Gerota's Prussian blue. Specimens of the thyroid were cleared by the Spaldeholz method, others cut serially in sections of 20, 50, 100, 150, and 200 μ, and still others by maceration and microdissection. The human thyroid was examined in the same manner, specimens being prepared at the operating table and from autopsies.

The lymphatic channels have their origin in the thyroid, in an anastomosing plexus which lies in the spaces between the follicles. This interfollicular plexus is composed of rather long, unbranched lymph capillaries which are in continuity with and connect dilated endothelial sacs or small pockets. These pockets were termed bursettes, and form with the lymph capillaries a closed lymphatic system in the thyroid gland. The bursettes and their communicating lymph capillaries lie exterior to the blood capillary plexus, which completely surrounds the individual follicles. The lymph channels drain large groups of follicles and are not distributed to each one individually. The interfollicular plexus anastomoses directly with an intraglandular reticulum, which runs along the septa of the gland, and connects also with a perivascular plexus, these latter connecting with an extraglandular plexus on the surface of the gland, from which the collecting trunks originate.

77. A histological study of a caudal appendage of a human infant. J. M. Robeson, Jr., and A. B. Dickey (introduced by H. E. Jordan), Laboratory of Histology and Embryology, University of Virginia, and Cora Donnell Hospital, Prescott, Arkansas.

This caudal appendage comes under the classification of Bartels as a 'free' tail and under that of Virchow as a 'soft' tail. It does not agree histologically with the only other tail which has been analyzed microscopically.

The human tail reaches its maximum development in the embryo between the 7-mm. and the 16-mm. stages. At 27 mm. the tail has atrophied except for a papilla. The tail-like appendages in adults are explained as persistent papillae.

The medullary tube extends to the tip of the tail at 6.6 mm., but at 15.5 mm. it is divided into two parts at the level of the thirty-second vertebra. The posterior part is atrophic and subdivides, the caudal element becoming the medullary coccygeal vestige. This caudal portion is believed to have given rise to the tail in this case. The tail contains an axial core of neuroglia arising of necessity from the epiblast. The persistence of the medullary coccygeal vestige in postfetal life demonstrates that this vestige may give rise to a neoplasm.

The occurrence of a few partially atrophied ganglion cells further supports the conclusion that this is a persistent medullary coccygeal vestige. The appendage corresponds to Mallory's description of a sacrococcygeal glioma.

78. *A study of the size of the sella turcica in white and colored males and females between the eighth and ninth years, as measured on flat x-ray films.* LAWRENCE T. ROYSTER AND MARGARET E. MORIARTY (introduced by H. E. Jordan), University of Virginia.

The skulls of 200 children between their eighth and ninth years were studied by means of flat x-ray films. There were fifty each, male and female, white and colored. The sella turcicas were measured in the anteroposterior diameter, the vertical diameter, and the interclinoid space. The difference was smaller than might be expected. The smallest average anteroposterior diameter was 8.08 mm. in colored females, and the largest average, 8.5 mm. in white males. The smallest average vertical diameter was 5.66 mm. in colored females; the largest average, 6.4 mm. in white males. The order of size was constant in both of these measurements: colored males, white males, white females, colored females.

The interclinoid measurements averaged least in colored females, 3.56 mm., and greatest in colored males, 4.28 mm.

A study was then made of the sizes of the sellae and calvaria by measuring the surface areas by means of a planimeter. In order of their sizes they were: colored males, colored females, white males, white females, and ranged from 0.430 sq.cm. to 0.489 sq.cm. The order of the size of the calvaria was as follows: white male, colored male, white female, colored female, and the size ranged from 166.6 sq.cm. to 184.5 sq.cm.

The ratio between the size of the sellae and that of the calvaria ranged from 1: 360 to 1: 411. The order was: white female, white male, colored male, colored female.

79. *The geometry of growth of the great or frontal fontanelle in man.* RICHARD E. SCAMMON, Institute of Child Welfare and Department of Anatomy, University of Minnesota.

The postnatal involution of the frontal fontanelle is a problem in developmental geometry. The area of the fontanelle may be regarded as a space bounded by four equal and tangent circles. By this hypothesis the area of the fontanelle is a function of its oblique diameter (approximately 1.25 times the diameter squared). This 'rational' expression has been compared with empirical results obtained from measurements of the fontanelles of 325 living infants of various ages. If these observations are summed for 0.5-cm. intervals of fontanelle diameter, the observed means are significantly different from the corresponding values obtained by the 'rational' expression. But when the observed medians for these same groups are compared with the corresponding values obtained by the 'rational' expression, the differences are not significant. The distribution of observations leads to the conclusion that, in spite of all efforts to the contrary, same rachitic individuals with enlarged fontanelles were included in this sample. The use of median values reduces the effect of these abnormal cases on the measures of central tendency, and, when this is done, the observed and geometrically calculated results are in essential agreement. These results tend to support the older views of Elsässer, Rohde, and Friedleben regarding the mechanics of growth of the calvarium, as opposed to the later opinions of Kassowitz and of Michael Cohn.

80. *Cellular proliferation in heterotopic spinal-cord grafts.* AURA EDWARD SEVERINGHAUS (introduced by S. R. Detwiler), Department of Anatomy, College of Physicians and Surgeons, Columbia University.

This investigation deals with the production of cells in isolated grafted pieces of embryonic spinal cord. Detwiler's spinal-cord grafts indicated the possible influence of descending and ascending fiber tracts upon cellular proliferation in the cord, and also offered experimental evidence in support of Coghill's observations that there are certain localized regions with extensive proliferation capacity where cell division takes place apparently independent of extraneous forces. In order to study this cellular proliferation in the absence of the possible rôle played by projection fibers, various units consisting of three or more segments from the cords of Amblystoma punctatum embryos (tail-bud stage) were grafted lateral to the host cord and at different axial levels of the embryos. These grafts had either normal or reversed anteroposterior orientation.

The experiments show that the transplanted cord is always larger than the normal. Moreover, it tapers off posteriorly and to a greater degree than the normal cord. Since the inherent tapering is maintained in the reversed grafts, it is opposite in direction to that of the normal cord. In fact, the grafts develop in all respects entirely independent of their orientation. A cellular hyperplasia, moderate in the ventral area, but very extensive in the dorsal region, is a constant feature. In consequence, the gray area becomes broadly U-shaped in cross-section, rather than pear-shaped as in the normal.

81. *Development of the sex glands of Calotes. I. Cytology and growth of the gonads prior to hatching.* C. S. SIMKINS AND J. J. ASANA, University of Tennessee School of Medicine.

The genital ridge of Calotes begins to form while the eggs are in the oviduct. In it are to be found two kinds of cells: small, spherical nucleated ones and large irregular nucleated, indefinite units in which are incorporated large granules resembling yolk. The latter units have been called the nurse cells and soon disappear from the fundament.

While the nurse cells are disappearing the sex cells arise from the smaller elements, the products of the germinal epithelium, at the close of the second week of incubation. The nurse cells are not confined to the germinal epithelium nor to the anlage of the gonad, but are to be found along the entire dorsal wall of the body cavity, even in that region which gives rise to the cortex of the suprarenal gland, hence cannot be looked upon as primordial germ cells.

The sex cells arise in situ by differentiation of somatic cells derived from the germinal epithelium. At the time of hatching there are gonads with a thick cortex and others with a thin cortex. It is suggested that the gonad with a thick cortex becomes an ovary and the one with a thin cortex gives rise to a testis.

82. The specific gravity of the brain of the male albino rat. C. G. **Smith** (introduced by C. C. Macklin), Department of Anatomy, University of Western Ontario.

The purpose of this study was to determine the density of the brain of the albino rat and follow the changes it underwent with age. The observations were made on seventy-four male and six female rats of the experimental colony strain of the Wistar colony.

The specific gravity was obtained by calculation from the weight and the volume of the brain freshly removed from the skull. For the determination of the volume sufficiently accurately for this work, a special apparatus was designed which permitted the reading of the volume of the brain to 0.001 cc.

The results show that the specific gravity which the brain has at birth, namely 1.029, is retained up to the age of fifteen days. At the age of five weeks it is at a maximum of 1.042, at seven weeks it is at a minimum of 1.036, and at nine weeks it is approximately at the density which it attains at the age of twelve weeks, viz., 1.040. The density at the age of one year is also 1.040, but at the age of two years it is up to 1.043. In the aged rats (three years old) the density was reduced to 1.038. The density of the female brain at the age of three months was the same as that of the male for the same age, viz., 1.040.

83. Effect of hypophysectomy upon the involution of the thymus in the rat. **Philip E. Smith**, Department of Anatomy, College of Physicians and Surgeons, Columbia University.

The thymus in rats hypophysectomized shortly before or after sexual maturity undergoes involution. Although its absolute decrease in weight is greater than in the normal controls, yet due to the stunting of general body growth which follows hypophysectomy, the percentage weight of this gland on body weight is nearly the same in the two types of animals. The reduction in the weight of the thymus commences immediately, even though the pituitary is ablated before the time at which thymus involution normally occurs.

The decrease in the weight of the thymus following the removal of the pituitary may not indicate any specific interrelation between these two glands, for conditions such as inanition and thyroid deficiency which are induced by hypophysectomy cause thymic involution. It has been generally found, however, that a failure of sexual development and an adrenal insufficiency either slow the involution of the thymus or cause a hyperplasia. In the hypophysectomized animal there is both a gonadal and an adrenal insufficiency which should produce a thymic hyperplasia. Some other factor, whether specific or general, however, is present after hypophysectomy which prevents a hyperplasia and induces involution.

84. The perforated and perforating flexor muscles in the feet of primates. **William L. Straus, Jr.**, Department of Anatomy, Johns Hopkins University.

The perforated flexor (flexor digitorum brevis) exhibits a tendency among primates to transfer its origin from the flexor tibialis tendon to plantar aponeurosis and calcaneus. Almost complete transference has occurred in man. Orang-utan approaches the human condition most closely. Conclusions as to genetic relationship cannot be drawn from degrees of transference. Loss of the tendon for toe V, extremely rare in lower primates, is more frequent in great apes than in gibbon and man.

The perforating flexors (fibular and tibial) are rather evenly distributed to all toes in prosimians—a primitive arrangement. In anthropoids the fibular flexor tends to supply toes I, III, and IV; the tibial, II and V—an arrangement foreshadowed in many monkeys (in orang the hallucal tendon is missing). Man and gibbon exhibit a more primitive condition, especially in the constant fibular supply to toe II, and relatively high occurrence of a tibial element in the hallucal tendon.

The quadratus plantae (closely associated with perforating flexors) arises primitively by a single head, as in monkeys, in which it is almost constantly present. The muscle has been lost entirely in prosimians and gibbons, most gorillas and orangs, and many chimpanzees. In man its absence is extremely rare, and it has developed a second head.

The anthropoid-ape arrangement of these important muscles does not appear to be ancestral to the human condition.

85. *Partial gigantism.* G. L. Streeter, Carnegie Laboratory of Embryology.

At the meeting last year, the author showed human specimens exhibiting defective development of limb-bud tissues. In contrast to those, a case will be described in which similar areas show an exaggerated response to growth stimuli, a case of partial gigantism limited to the right forearm and hand and to the left index finger and thumb.

86. *The meninges and fourth-ventricle apertures of the albino rat.* R. M. Strong and Thomas P. Hickey, Department of Anatomy, Loyola University School of Medicine.

The meninges are thin, as would be expected in a small animal. They will be described with the aid of lantern slides. The fourth ventricle is relatively very narrow and its space is largely filled with chorioid plexus caudal to the relatively large lateral recesses. Definite lateral apertures into the subarachnoid space occur, but no posterior median aperture was found. Relatively large processes of chorioid plexus extend through the lateral apertures into the lateral subarachnoid space.

87. *Use of the x-ray in embryology.* Walter E. Sullivan, Anatomical Laboratory, University of Wisconsin.

Some three years ago, small specimens were immersed in, and large ones injected with, solutions of metals that were of sufficient atomic weight to be effectively absorptive of x-rays. Being somewhat familiar with the use of iodized oil, we began with the iodides. Frogs were used as specimens and the first roentgenograms gave good visualization of the eyes. Later compounds of lead, mercury, and silver were used and it was found that bichloride of mercury and lead nitrate were easily controlled. The former combines readily with the central nervous system and the viscera, the latter with recently formed bone. Solutions of various concentrations were used, but relatively weak ones were the most satisfactory. Among the obvious uses of the method is the examination of a large number of specimens in a relatively short time, with results fully as satisfactory as with cleared specimens. The saturation point with many common fixatives can be determined.

88. *Further observations on the production of supernumerary limbs in Amphibia.* F. H. SWETT, Department of Anatomy, Vanderbilt University School of Medicine.

The high incidence of supernumerary limb formation in the course of experimental studies upon the development of these appendages in Amphibia has led to considerable speculation as to the manner of their institution. Mangold has suggested that the extra limbs, which have been reported by Harrison and others in Amblystoma punctatum after harmonic grafts to the flank, may have been 'induced,' and not developed directly from the cells contained in the transplant. He also suggested that this might be put to the test by use of heteroplastic transplants. This procedure was carried out during the present season, using three species of Amblystoma as reciprocal donors and recipients. A considerable number of reduplicated limbs resulted. If the supernumerary components were 'induced,' the expectation was for their characteristics to be those of the recipient species. In all cases, however, both components showed the characteristics of the donor species. This has been taken to indicate that the extra limbs, as well as the primary members, developed from the grafts, and were not primarily the result of any induced activity on the part of the cells in the body wall of the recipients. The results of experiments did not preclude the possibility of some contribution to the limbs by cells of the recipient, but there was no evidence that this occurred.

89. *An aqueous extract of the suprarenal cortex which preserves the life of adrenalectomized cats.* W. W. SWINGLE AND J. J. PFIFFNER, Biological Laboratory, Princeton University.

An aqueous extract of suprarenal cortex is prepared by extracting the ground tissue (beef) at room temperature successively with 95 per cent alcohol and 80 per cent alcohol. The extractives are concentrated in partial vacuo at a low temperature and the residues extracted with benzene. The benzene is removed in partial vacuo at a low temperature and the residue extracted with acetone. The residue after removal of the acetone is distributed between 70 per cent alcohol and petroleum ether. The alcoholic phase is washed, the petroleum-ether washings combined, and the petroleum ether removed. The residue is again subjected to the distribution procedure. The petroleum-ether washings are washed with 70 per cent alcohol. The alcohol is removed, and the aqueous concentrate sufficient distilled water is added to obtain the desired volume. One cubic centimeter of extract represents 30 grams of fresh cortex. Clarification and sterilization are effected by Seitz filtration.

Adrenalectomized cats treated with the aqueous fraction remain in normal healthy condition and cannot be distinguished from unoperated animals. We have no difficulty in keeping animals fifty or sixty days, and have several on hand which are now in their eightieth day of survival and show no symptoms. Several animals were sacrificed on the fiftieth day and careful search revealed no accessory cortical tissue. The dosage used is 0.5 to 1 cc. per kilogram of body weight daily subcutaneously.

90. *On determining temperature changes in the respiratory tract.* R. J. TERRY AND GEORGE A. SEIB, Department of Anatomy, Washington University, St. Louis.

In a previous search for evidence of fluid in the mammalian lung, a slight but constant difference in the specific gravity of the blood of the two sides of the heart (cat) was discovered. Pursuing this problem, it was found necessary to make investigations of certain physical properties of respiratory air. Study of temperature changes in several parts of the respiratory tract have been undertaken. To make these determinations with a minimum of physiological disturbance, the thermo-electric method was used.

Two thermocouples (insulated iron-constantin wire) were employed: one kept at uniform temperature, the second introduced into the respiratory tract. In the latter case the wires were passed through a small ureteral catheter, their ends soldered together at the tip of the catheter and cemented in place, giving a narrow, flexible, and waterproof thermocouple. Temperature changes to 0.01°C. were registered.

This thermocouple was passed into the external nares of an anaesthetized cat, pushed into the nasopharynx, led into the larynx, thence into the lung, the distance of insertion having been previously determined and marked on the catheter. Galvanometer deflections were recorded with the thermocouple in the lung, and at several selected levels (checked by autopsy) reached by withdrawing the catheter.

91. *Estimation of the cutaneous-nerve areas of the forearm and hand.* I. MACLAREN THOMPSON, VERNE T. INMAN, AND BERNARD BROWNFIELD (introduced by Herbert M. Evans), Department of Anatomy, University of California.

By an electrical technique the cutaneous area supplied by a nerve is rendered insensible to light touch and is thus outlined; the outline, transferred to paper, is planimetered.

Measurements upon sixty-five limbs of thirty-five male medical students (thirty pairs + two right + three left) have yielded the following results (square centimeters); the appended estimations are standard errors (sigmas), not 'probable errors.' Median: mean, 218.4 ± 3.6; standard deviation, 27.9 ± 2.5. Ulnar: m., 219.0 ± 4.3; s.d., 35.0 ± 3.1. Radial (superficial ramus): m., 161.6 ± 7.9; s.d., 63.4 ± 5.6. Medial antebrachial cutaneous: m., 361.2 ± 8.2; s.d., 66.4 ± 5.8. Lateral antebrachial cutaneous; m., 243.0 ± 5.4; s.d., 43.4 ± 3.8. Dorsal antebrachial cutaneous: m., 262.4 ± 5.8; s.d., 47.0 ± 4.1. Because of a technical difficulty, the proximal boundary of the radial must be assigned by an arbitrary rule.

These are gross areas. The following will exemplify our estimates of the numerous overlap areas in this region. Medial and lateral antebrachial cutaneous: m., 46.5 ± 2.6; s.d., 20.9 ± 1.8. Dorsal and lateral antebrachial cutaneous:

m., 56.7 ± 2.7; s.d., 22.0 ± 1.9. Dorsal and medial antebrachial cutaneous: m., 44.2 ± 2.1; s.d., 16.6 ± 1.5.

A detailed account of the technique and of our treatment of its experimental error, together with an analysis of the results, will soon be published.

92. A quantitative analysis of the changes and recovery of form of the human head at birth. Leon J. Tiber (introduced by Richard E. Scammon), Department of Anatomy and Department of Obstetrics and Gynecology, University of Minnesota.

The material consisted of full-term healthy newborn infants—107 males and 111 females. The measurements included: standing and sitting heights, weight, and twenty-one head dimensions. Measurements were made within six hours of birth and each day thereafter to ten days.

The median, semi-interquartile ranges (absolute and relative) and differences between birth values and preceding-day values and the grand mean of each dimension for the period were determined.

Results: Changes in head dimensions in the ten days range from +1.0 to —0.1 cm. Two dimensions show no change. Computed in terms of standing height, approximately half the head dimensions gain and half show a loss in the period.

The corresponding relative changes range from +6.8 to —0.65 per cent. All dimensions attain their mean for the ten-day period on the fifth and the eighth day. Cranial-vault and face dimensions attain this average on the fifth or sixth day. Those involving the skull base attain this value about two days later. The curves of six dimensions show little or no change in the period, three show a distinct decrease and recovery, eleven show a consistent increase, and one is irregular. Males exceed females in absolute dimensions. There are no consistent sex differences in relative size of dimensions. The neonatal changes of the two sexes are highly concomitant, the rank correlation being 0.8046 ± 0.0520.

93. The effect of sympathetic denervation of skeletal muscle. S. S. Tower, Department of Anatomy, Johns Hopkins University.

Various lengths of the thoracic-cervical sympathetic chain were removed on side from a series of adult dogs, cats, and goats. In animals surviving two months to two years, specimens of muscle from the fore paw, lower arm, and upper arm were prepared according to Boeke's Bielschowsky technique; the intensity of staining, clearness of striation, number and size of nuclei, and the development of connective tissue were studied and the diameter of one hundred randomly chosen fibers in each specimen measured. The muscle of the sympathetically denervated side was in all these respects indistinguishable from the normal. Also, hypolemmal nerve endings other than those of medullated nerve fibers were not encountered in either operated or normal material.

The effect of similar sympathetic denervation on the growth of muscle was studied in a series of kittens operated within the first ten days of life and surviving as long as one year. These muscles have not yet been examined histologically, but careful examination during life brought out no differences in the growth or activity, in the muscle bulk, strength, or tone on the two sides.

Therefore it seems that neither in the adult nor in the considerably more taxing period of growth does the sympathetic innervation of normal muscle exert any trophic effect on the muscle fiber which is reflected visibly.

94. The origins of branches of the axillary artery in whites and in American negroes. MILDRED TROTTER, J. L. HENDERSON, H. H. GASS, R. S. BRUA, S. WEISMAN, H. AGRESS, G. H. CURTIS, AND E. R. WESTBROOK, Department of Anatomy, Washington University, St. Louis.

Observations on the origins of the named branches which are expected to arise directly from the axillary artery were made on a series of 192 cadavers. In approximately 50 per cent of the white male and female and negro male groups the branching of the axillary artery conformed to the usual text-book description, while the negro female group showed the highest incidence of cases having the anterior and posterior humeral circumflex arteries arising from the axillary by a common trunk.

In the group which showed variations from the 'normal' arrangement (approximately 50 per cent), of about equal frequency were the conditions of the lateral thoracic artery arising from the subscapular and the anterior and posterior humeral circumflexes arising by a common trunk. The posterior circumflex arising from the subscapular was the variation next in frequency, and in only a few cases did the lateral thoracic arise from the thoraco-acromial.

The majority of bodies showed a similar condition, whether 'normal' or with variation, on the two sides, but when only one side presented variations it was more often the right than the left.

No racial differences were observed in the male groups.

95. The architecture of the tunica media of arteries. JOSEF D. WEINTRAUB, (introduced by O. V. Batson), Graduate School of Medicine, University of Pennsylvania.

Although the histologic components of arteries have been known for decades, the arrangement of these elements is only at present being studied. To do so microscopically involves tremendous numbers of serial sections and reconstructions. By means of the splitting method and dissections of fresh and of fixed (embalmed) arteries, some partly macerated, coupled with histologic examination, it is possible to determine the architecture of whole portions of the arterial tree.

The splits in the tunica media aortae are in the main nearly circular. This tunic, thin at the sinus aortae, becomes thicker rather suddenly along three curved lines connecting the commissurae valvularum semilunarium. These lines have their concavity toward the valvulae semilunares, and bound the sinus superiorly. Thence upward for several centimeters the fibers are disposed in arcs, concavity downward, between one commissure and the next. Not all the fibers are in arcs, some are circular about the vessel. The same arrangement occurs in the corresponding part of the arteria pulmonalis. These arched fibers of the vessels, under tension, aid in supporting the downward thrust on the valvulae semilunares.

At the beginnings of the various branches of the aorta there are characteristic groupings of the fibers of the tunica media.

The splits at the attachments of the neonatal ductus arteriosus are similar to those at the attachments of the ligamentum arteriosum.

96. *Origin of the deep and circumflex femoral group of arteries.* G. D. Williams, R. J. Sindelar, J. C. Peart, N. A. Martin, L. R. McIntire, C. H. Martin (introduced by R. J. Terry), Department of Anatomy, Washington University, St. Louis.

In 107 thighs of American white males and fifty-nine of American negro males dissected, determinations were made of the origins of the members of the deep and circumflex femoral group of arteries. Differences in incidence of the various types of origin of these arterial branches were found to exist between right and left sides of the body, and between the white and the colored males in the series. In any statement concerning frequency of occurrence of the several varieties of origin of the vessels in question, it is necessary, therefore, to segregate the data according to side of body and according to race, or at least to take the factors of side of body and of race into account in the statistics relating to such a distribution. This principle has been followed in presentation of the statistical table of this paper.

97. *A study of the lumbar enlargement in chicks following early isolation from the rest of the nervous system.* Roy G. Williams (introduced by E. R. Clark), Department of Anatomy, University of Pennsylvania.

In order to analyze some of the possible growth-regulating factors of the nervous system, the lumbar enlargement of the spinal cord of chick embryos was isolated in situ by cutting off the tail and by removing several segments of the spinal cord immediately cephalad to the lumbar segment and inserting a piece of eggshell in the gap. The twenty-third to the twenty-ninth segments, inclusive, with their anterior and posterior roots, were left intact. The operations were performed at fifty-six hours of incubation, and afterward the eggs were returned to the incubator and allowed to continue development.

In operated chicks which hatched, the legs were held in rigid extension, but they showed a certain degree of response to external stimulation.

Serial sections of such operated specimens, as compared with control chicks, showed a 13 per cent reduction in the size of the gray matter, a 43.9 per cent reduction in the size of the white matter, and a 41 per cent reduction in the number of cells. The distribution of cells had not been significantly affected. There was no appreciable change in the size of the motor horn cells.

98. *Correlation in development of the heart and liver in chorio-allantoic grafts of whole chick blastoderms.* B. H. Willier and Mary E. Rawles, Department of Zoölogy, The University of Chicago.

The differentiation of heart and liver has been studied in chorio-allantoic grafts of whole pellucid areas of chick blastoderms of stages before (primitive streak) and after (head process to ten somites) the heart rudiments are formed. The liver primordium arises much later than the oldest stage transplanted. The grafts grew for eight or nine days. Of forty grafts examined, the heart has developed in thirty-two and the liver in twenty-four cases. Typically the heart is a muscular, spindle-shaped body without chambers, but having a lumen containing blood. It is often found pulsating. The liver is a histologically normal body without organization into lobes or gall bladder.

The liver and heart are in intimate contact (sixteen grafts) or closely associated (fifteen grafts)—a relationship similar to that existing between the entoderm and heart rudiments of an early blastoderm. Two hearts have developed in eight grafts. Of these, seven show two livers, each in contact or closely associated with a heart. In these cases, apparently the mesentoderm of the amniocardiac vesicles has been prevented from uniting into a single median heart. In eight grafts heart has developed without liver. The liver, on the other hand, has never developed in the absence of the heart.

The results indicate, 1) the presence of right and left areas of prospective liver cells in the entoderm situated ventral to the heart rudiments and, 2) the dependence of the liver upon the heart in its origin.

99. *The earliest fetal movements in the cat correlated with the neurofibrillar development of the spinal cord.* William F. Windle, Northwestern University Medical School.

Motility was first observed in embryos 17.5 and 18.5 mm. long which had been quickly removed from cats decerebrated over two hours previously. The circulation was intact and no anesthetic interfered. Movements could be determined only in the region of the neck and pectoral girdle. They appeared as worm-like, total waves proceeding caudad from an indefinite point in the neck region. They produced ventrolateral flexion of the head and upper trunk, accompanied, in the older specimens, by very slight rotation of the head and total flexion of the fore legs.

Pyridine-silver preparations of these embryos demonstrate the neurofibrillar structure of the central nervous system. Primitive reflex arcs are distinguishable, afferent, association, commissural, and efferent neurons appearing in proper relationship to each other. More are seen in the cervical than in the lumbar cord where collaterals from the posterior sensory column are very few in number. All other elements of a simple arc appear before these collaterals, which are first distinguishable in the upper cervical segments of 10-mm. specimens. There they seem to arise from sensory components of the accessory nerve which have entered into the formation of the caudal end of the tractus solitarius.

The earliest movements appear in regions where primitive reflex arcs were first distinguishable and not until some time after these have formed.

100. *The female reproductive tract of the gorilla, with a comparison of those of other primates.* George B. Wislocki, Department of Anatomy, Johns Hopkins University.

The present study deals with the female reproductive tracts of three adult gorillas; these have been compared with equivalent reproductive tracts of man, other anthropoid apes, and monkeys. There exist in the Simiae tremendous species differences in the configuration of the reproductive organs, but the Simiae fall, nevertheless, into definite groups on the basis of the morphology of the reproductive tracts. The material has been compared from the standpoints of the external and internal genitalia and placentation. In reference to all of these divisions, the three great apes—gorilla, chimpanzee, and orang-utan—show close relationships. Man and the gibbon, although more closely related to the great apes than to the catarrhine or platyrrhine monkeys, show marked dissimilarities from the three great apes. The external genitalia of man are unlike the apes or catarrhines and resemble the more primitive platyrrhine monkeys. In regard to internal genitalia, however, man resembles markedly the great apes and in placentation his identity with the group is almost absolute. The gibbon shows affinities in different characters to the great apes, to man, and to the catarrhine monkeys.

Man's similarity to the gibbon in certain respects indicates that he is related as intimately to the most primitive of the anthropoid apes as to any of the others. This fact, as well as man's isolation in reference to external genitalia, suggests that his genetic bonds with the existing apes are more remote than is usually assumed.

101. The adrenal cortex and blood sugar. R. L. ZWEMER, J. M. SMITH, AND M. SHIRLEY (introduced by P. E. Smith), Department of Anatomy, College of Physicians and Surgeons, Columbia University, and the Biological Laboratory, Cold Spring Harbor, Long Island, New York.

Lowered blood sugar following removal of the adrenals has generally been attributed to loss of medulla and adrenin. We have shown that cats retaining only the cortex survive normally. Prof. W. W. Swingle, therefore, suggested that blood sugar be determined in animals so prepared. Blood was withdrawn from ear vein or heart and analyzed by the Folin-Wu or Schafer-Hartman methods. The normal range was between 84 mg. and 166 mg. per 100 cc. (twenty-nine determinations averaged 109.1 mg.). Removal of one adrenal medulla or one entire adrenal had no permanent lowering effect, the averages being 119 mg. and 91.3 mg., respectively. Animals possessing the cortex only of one adrenal appeared normal and had blood sugars ranging between 70 mg. and 150 mg. (twenty determinations averaged 97.4 mg.). The lowest figures were derived from samples taken when the animals had transient adrenal insufficiency. Removal of the cortex is followed by lowered blood sugar. The decrease appears with other insufficiency symptoms and the sugar varies between 44 mg. and 87 mg. (twenty-three samples averaged 66.3 mg. per 100 cc.). In considering the effect of the adrenal on blood sugar, the rôle of the cortex should not be overlooked.

PAPERS TO BE READ BY TITLE

1. The induction of egg laying in Triturus viridescens by heteroplastic pituitary-gland grafts. A. ELIZABETH ADAMS, Zoölogy Department, Mount Holyoke College.

Transplantation of anterior lobes of pituitary glands of male frogs (Rana pipiens) into seven adult Triturus induced egg laying in all, while frog posterior lobes or muscle placed in nine animals gave negative results. The numbers of grafts implanted before egg laying occurred and the number of eggs laid per animal varied. These numbers appear to be correlated with the size of the animal or its previous egg-laying activities (some had already laid eggs following homoplastic pituitary grafts), but the number of animals used is too small to give conclusive data. There may also be some differences in the 'taking' of the transplant. To date, totals of eggs laid per animal run as follows: 11 (13 anterior lobes engrafted over a period of 28 days); 12 (4 lobes, 11 days); 13 (6 lobes, 14 days); 20 (14 lobes, 15 days); 29 (10 lobes, 12 days); 40 (2 lobes, 2 days); 50 (2 lobes, 1 day). There is a slight possibility that these totals may not represent the full number of eggs laid, because in one case an animal is known to have eaten two eggs.

Previous experiments (Adams, '30) have demonstrated induction of egg laying in Triturus by homoplastic pituitary grafts (whole glands, anterior lobes). These indicate that heteroplastic grafts (anterior lobes) will act similarly.

2. *Development of eggs laid by Triturus viridescens following pituitary grafts.* A. Elizabeth Adams, Zoölogy Department, Mount Holyoke College.

Nearly 600 eggs have been laid out of season (December 14th to March 9th) by nine specimens of Triturus which have received homoplastic (whole gland or anterior lobes from male Triturus) or heteroplastic (anterior lobes from male Rana pipiens) pituitary-gland grafts. In the case of five animals, a small proportion of the eggs underwent some development, and at the present date (March 9, 1930) twenty-one embryos in various stages are still alive. In nine of these the mouths are beginning to open. Since four of the females laying the eggs have been separated from males since the middle of December, the developing eggs must have been fertilized by sperms present in the spermatheca previous to that time (possibly from the fall mating season). Examination of the spermathecae of these animals has not yet been made. Parthenogenesis may possibly have occurred, but this seems rather unlikely.

3. *Exposure of oestrus-producing ovarian hormone to light rays.* Edgar Allen and E. L. Clausen, University of Missouri and University of Rochester.

Destructive action of massive doses of ultraviolet rays and sunlight upon follicular or oestrus-producing ovarian hormone has been reported.

Following the demonstrations of curative effects of ultraviolet light upon rickets, one of us (E. L. C.) has dedmonstrated a definite effect of infrared light upon rickets. This effect is, briefly, one of producing growth and significant prolongation of survival of rats on a rickets-producing diet without, however, other alleviation of the disease.

Following these results, several samples of follicular hormone ('Amniotin,' Squibb) were rayed under filters which transmitted the following limited parts of the spectrum: ultraviolet and green, green, blue, orange red and infrared. Control samples were exposed in the dark room at the same time. Times of exposures varied from two to six hours. The potency of the samples was then tested by series of injections into ovariectomized rats.

It was found that twenty times the exposure to various light rays required for the effect of infrared light on rickets in rats had very little if any destructive power upon this ovarian hormone. Other selected light waves seem to have only a slight destructive effect. These results are of a preliminary nature, but they show clearly that this hormone is relatively stable under exposure to isolated segments of the spectrum which exert definite photochemical effects upon living animals.

4. *Exceptional menstrual periods induced in ovariectomized monkeys by oestrus-producing ovarian hormone.* Edgar Allen and Dan D. Baker, University of Missouri.

Two ovariectomized adult monkeys were given twenty-four days' continuous treatment with oestrus-producing ovarian hormone. This active substance ('Amniotin,' Squibb) was administered by gelatin pessaries inserted into the vagina twice daily. The total dose exceeded 1160 rat units per animal. Maximum coloring of the 'sexual skin' was induced.

A 'latent' period of six days followed cessation of hormone administration. Menses began in both monkeys on the sixth day after the last dose. Duration of menses was nine days in one animal and seven in the other, or nearly twice the usual period. The flow was more profuse than noted in normal monkeys or during experimental menses previously induced by hormone injections in ovariectomized monkeys.

During hormone administration it was possible to recover some of the active material excreted in the urine.

It would seem that administration of hormone in gelatin pessaries by the vaginal route is more effective than subcutaneous injections of water preparations. Surely it should prove less objectionable to patients.

5. *Sexual coloring in monkeys.* Edgar Allen and D. C. Robertson, University of Missouri.

During periods of sexual activity both male and female monkeys experience reddening of the skin of the genital regions, buttocks, and thighs. This reddening of the 'sexual skin' in the female fades after ovariectomy and can be induced again by injections of lipoid extracts of ovary and placenta.

A normal male between seven and eight years of age developed bright coloring of the 'sexual skin.' The testes of this monkey (of body weight of less than 30 pounds) were two and one-half times the average weight quoted in anatomy texts for those of adult man. This monkey was castrated toward the end of summer. Within two months, the color had faded to a very light red.

Then injections of oil solution of lipoid extracts of human placenta were given twice daily for forty-eight days, a total of more than 400 rat units of active material being injected. The color deepened considerably by the ninth day of injections. By the thirty-fourth day, it had developed to a degree equivalent to that at the time of castration and it continued so until the end of the experiment. There was also a slightly swollen area at the base of the tail.

This experiment seems to be a clear demonstration that the reddening of the 'sexual skin' of the male monkey can be induced by sex hormone from female sources.

6. *Anomalous mouse embryos at end of first day in life of embryo proper (eight days, gestation age).* Ezra Allen, New York Homeopathic Medical College and Flower Hospital.

Exceptionally small embryos show on sectioning certain defects, some of which are interpreted as lethal. Development in some embryos has been apparently normal through part of the proembryonic history, when degeneration has set in. These embryos show degeneration chiefly internally in the region of developing membranes. In other embryos there is evidence of abnormal segmentation, resulting in nearly separate twins of unequal size, or in a nearly normal but small embryo, attached to which are bodies that resemble incomplete proembryos, all within a single yolk sac. Other embryos are apparently normal, but much retarded. Other cases await further study for classification. The present analysis helps to explain the extreme variation in weight at this age, and to account for some of the prenatal mortality.

7. *Tissues from a 52-cm. (C.R.) human fetus stained by various methods.* EZRA ALLEN, HADLEY KIRKMAN, AND IRENE KIRKMAN, New York Homeopathic Medical College and Flower Hospital.

The fetus died shortly after delivery and was prepared immediately by washing out the blood with normal salt solution and injecting thoroughly with Bouin's fixing fluid. Sections of the following organs will be shown: cerebellum; digit, for bone marrow and subcutaneous tissue elements; penis at different levels; thyroid, trachea, and esophagus; testis. Tissues have been stained by two or more methods, each designed to demonstrate a part to the best advantage, or for comparisons.

8. *A study of degeneration of the lateral-line sense organs in Ameiurus nebulosus (Les).* C. H. ALVEY (introduced by A. B. Dawson), New York University.

The lateral-line branch of the vagus nerve was cut dorsal to the lateral line, directly below the anterior edge of the dorsal fin. One or two millimeters of the nerve were removed and examined microscopically. Serial sections of the integument were studied from fish killed 5, 7, 10, 13, 16, 22, 27, and 35 days after the operation. In each case the integument from the unoperated side served as a control. Fixation in Schaudinn's fluid was followed by haematoxylin-eosin. The sense organs in the sections examined five and seven days after operation showed an apparent decrease in the number of sense cells present as well as a looseness in structure. Sense organs examined in tissue removed from fish at longer intervals after the operation compare favorably with the controls. After the seven-day period, there is apparently a recovery and return to normal. Since the nerve fiber has been shown to regenerate only after approximately thirty-five days, it is suggested that the changes occurring in the seven-day interval after operation are the result of an injury reaction, and further that the maintenance of the definitive differentiation of the lateral-line sense organs in Ameiurus nebulosus (Les) is not completely dependent upon the integrity of its nerve supply. Further work is now in progress checking injury reaction alone.

9. *Compatibility of oestrin and progestin.* R. ANGELVITZ AND EUNICE F. STERLING (introduced by G. W. Corner), Department of Anatomy, University of Rochester.

The following experiments were undertaken to determine whether or not the corpus-luteum extract of Corner and Allen inhibits the action of oestrin. This lipid extract contains from 0.25 to 0.5 rat unit of oestrin in addition to 2 rabbit units of progestin per cubic centimeter.

1) Four spayed rats were each injected with 0.5 cc. corpus-luteum extract; 2) four, with a mixture of 0.5 cc. corpus-luteum extract and 1.5 r.u. of Estrogen. All other rats received separate, but simultaneous injections. 3) Three received 0.5 cc. corpus-luteum extract and 1.5 r.u. Estrogen; 4) five, 0.5 cc. corpus-luteum extract and 0.75 r.u. Estrogen; and, 5) four, 0.5 cc. corpus-luteum extract and 0.66 r.u. Estrogen. (Estrogen injections were made in three fractional doses at five-hour intervals.)

All rats of the first group showed negative reactions; all those of the second and third, three of the fourth, and one of the last, positive. All other rats indicated partially positive reactions. Our results indicate that there is no antagonistic action of the corpus-luteum extract to that of oestrin.

10. *Neurological interpretation of fetal behavior: The progressive increase of muscular activity in albino-rat fetuses.* A. W. Angulo y González, The Wistar Institute of Anatomy and Biology.

Motion-picture records have been obtained for the purpose of showing the typical stages in the development of muscular activity in albino-rat fetuses from the time when the first movement appears to a few hours before birth.

In previous communications to the Association, preliminary reports were made of the possible neurological interpretations of these various stages. These records are now offered as an illustration of such stages, and should be so interpreted.

The following stages are represented:

1. Lateral flexion of the trunk.
2. Flexion of the trunk with movement of the fore limb.
3. Extension of the trunk.
4. Maintenance of contractions.
5. Beginning of emergence of independent movements heretofore appearing as subordinated movements, also specific reflexes and postural reaction.

With each one of these typical movements there is a series of allied movements that appear concurrently and which are considered in the course of this presentation.

Incidentally it is seen that all these movements, including specificity of reflexes and postural reaction, occur many days before myelination takes place.

11. *An early menstrual repair stage.* G. W. Bartelmez, Department of Anatomy, The University of Chicago.

A human uterus removed on the fifth day after the onset and just after the end of a flow presents transitions between a wound surface and regeneration to the extent of the formation of surface epithelium with granulation tissue beneath it. The evidences of the preceding menstruation are the diffuse superficial hemorrhage, the pyknotic nuclei and fat granules in many gland and stroma cells, the macrophages, the granular mitochondria in some gland cells (all transitions are to be found between these and the long filaments characteristic of other phases of the cycle). Unlike menstruation stages, many capillaries can be demonstrated in sections. Correlated with this are the indications of repair, viz., the new epithelium over most of the surface, numerous mitoses especially in the gland cells, areas with reorganizing superficial stroma, and, in addition, the presence in some locations of Sekiba's zone of highly vascular granulation tissue. Different regions of the mucous membrane show different stages of development; such variability is to be found at all stages of the cycle, especially during menstruation.

12. *Golgi bodies, vacuome, and mitochondria in the salivary glands of the Chironomus larva.* H. W. Beams and J. B. Goldsmith (introduced by H. E. Jordan), Laboratory of Histology and Embryology, Medical School, University of Virginia, and the Department of Zoölogy, University of Wisconsin.

Our observations confirm the recent findings of Krjukowa ('29) on the specific character of the Golgi material in the salivary glands of the Chironomus larva and are therefore in disagreement with the findings of Parat and Painlévé ('24).

The Golgi material in the salivary glands of the Chironomus larva is present as discrete bodies having the form of crescents, rings, and rods. These are evenly deposited throughout the gland and show no marked variation in number or in form at different stages of the physiological activity of the gland.

The mitochondria are present in the form of filaments frequently concentrated in the area surrounding the nucleus and at the periphery of the cell.

Neutral-red staining was never observed to color the Golgi bodies. It is suggested that the neutral-red bodies may represent the secretory material. However, it is clear that whatever the significance of the neutral-red bodies, they are not Golgi material. Accordingly, this evidence supports the view that the Golgi apparatus, mitochondria, and neutral-red bodies are morphologically distinct structures in the cell of the salivary glands of the Chironomus larva.

13. *Some measured characters of Old Virginians.* Robert Bennett Bean, School of Anatomy, University of Virginia.

This study includes the stature, sitting height, and sitting-height index of 1752 Old Virginian males and 817 Old Virginian females from 6 to 60 years of age. The male stature is greatest at the ages between 17 and 20, because nearly all persons at these ages were students. The increment of the male stature is greatest between 9 and 10, with lesser increments about 7, 12, and 15, and the least increment between 13 and 14. The stature between 21 and 60 is about the same, around 173 cm. The female stature is greatest at 21 to 25 years, after which it is between 161 and 162 cm. The increment of the female stature is greatest between 11 and 12, although it is greater between 7 and 11 than the male, and there are lesser increments about 7 and 10 and the least increment is between 13 and 14. The male sitting height has its greatest increments about 7, 9 to 10, and 15, with the greatest about 9 to 10, and the adult condition is reached about 18, after which it remains the same, about 91, until the age of 60. The female sitting height has its greatest increment between 11 and 12, although it is great immediately preceding that, as well as about 7 and again about 20, after which it remains at about 86.5 cm. until 60 years of age. The sitting-height index of the males decreases until the age of 13, and then increases until the age of 50, with a slight decrease thereafter. The female sitting-height index behaves in a similar way. The index is the same in the two sexes before 10 years, but thereafter it is greater in the females, especially about 15, when the difference is nearly 2 points. In the adult the index in males is about 52.5, and in females, 53.5.

14. *An experimental study of opsonins in cat serum. I. Effect of ultraviolet radiation.* E. W. Blanchard (introduced by W. W. Swingle), Princeton University.

Normal cat serum, after being separated from the cells, was divided into two portions. One portion was irradiated, the other part served as the control. The source of radiation was a Hanovia mercury arc in quartz and the serum was exposed at a distance of 10 inches from the lamp. During all the experiments the temperature was controlled in order to rule out any heating effect.

Accurate dilutions of both the irradiated and the control serum were made. Cat leukocytes were obtained by intraperitoneal injection of 0.9 per cent NaCl and subsequent withdrawal of the peritoneal exudate. These cells were washed and suspended in a small amount of salt solution. Collodion particles, which have an acid-fast staining reaction, were used as the substance to be ingested. One-tenth cubic centimeter of serum, or its dilution, 0.1 cc. of leukocytes, and 0.1 cc. of collodion suspension were placed in small tubes which were corked and agitated slowly for fifteen minutes. After spreading and staining, counts were made and the opsonic activity expressed in terms of per cent of phagocytic cells.

Exposures of various lengths were made. One of ten minutes' duration will markedly diminish the opsonic activity of the serum and the results clearly indicate that a half-hour exposure will practically completely destroy the opsonin present in normal serum.

15. *An experimental study of opsonins in cat serum. II. Effect of bilateral adrenalectomy.* E. W. Blanchard (introduced by W. W. Swingle), Princeton University.

It has been noticed by various investigators that cats after the removal of their suprarenal glands show a markedly decreased resistance to various infections. The opsonic activity of such cats was studied.

The actual method of determining the opsonic activity has been outlined in the first section of this study. The cats were operated by the same individual and the two glands were removed separately, the cats having completely recovered from the first operation before the removal of the second gland. The operated animals were bled at frequent intervals and the opsonic activity of the serum determined.

The results show clearly that the opsonin content of the blood begins to drop about two days following the removal of the second gland. The experimental cats lived, on the average, about six days, and the opsonin had almost completely disappeared by the fifth or sixth day. Double-nephrectomized cats showing symptoms similar to those of complete cortical deficiency were also used and the serum of these animals showed no change in opsonic activity even at the terminal stage.

Double-adrenalectomized cats treated with an active extract of the suprarenal cortex (Swingle and Pfiffner) show normal opsonic activity. This would indicate that it is the cortical portion of the gland which is concerned in the maintenance of the normal defense mechanism.

16. *Replantation of the eye in the albino rat.* CHARLES BRECK (introduced by L. S. Stone), Department of Anatomy, Yale University School of Medicine.

An anatomical study was made of the gross and microscopic conditions of replanted eyes in sixty albino rats ranging in age from seventeen days after birth to the adult. Ten additional animals with only partial enucleation of the eyeball (a small tag of conjunctiva being left intact in an attempt to maintain the blood supply) were studied as controls. Serial microscopic sections showed early degenerative processes in all the retinal layers during the first twenty-four, forty-eight, and ninety-six hours after operation. Twenty-three and eight-tenths per cent of the grafts gave evidence of a reestablished circulation but within two months, 93.2 per cent either underwent complete resorption or persisted as small fibrous stumps. The same conditions were recognized in the controls. In only 6.8 per cent a small spherical eyeball persisted, in one case as long as 357 days after operation. Microscopic studies showed a complete replacement of the nervous structures by fibrous connective tissue and in no case was there an optic nerve connecting the eye with the brain. The corneas of these bulbs became thickened and vascularized very early, and failed to regain subsequently their original transparencies. The lens, although large in proportion to the entire organ, decreased in size. It retained its epithelial capsule, but its interior became less distinctly laminated than is found normally.

17. *The process of sex transformation in parabiotic Amblystoma. II. Transformation from male to female.* ROBERT K. BURNS, JR., Department of Anatomy, University of Rochester.

In twenty-nine cases of parabiotic Amblystoma tigrinum larvae, preserved at varying ages, five pairs showed the gonads of the male in process of transformation to ovaries. This transformation is made possible by the existence in the young normal testis of a peripheral germinal epithelium or 'male cortex,' homologous with the germinal epithelium or true cortex of the ovary. In a certain number of cases this male cortex, under stimulation by the hormones of the female partner, may grow rapidly; while concomitant regression of the medullary region of the testis occurs. These processes may result in a transformation so complete that large sections of the gonad are indistinguishable from normal ovary, while in other sections transformation is still in progress.

The fact remains that transformation from male to female occurs in a relatively small minority of cases, while in all others the male is dominant. Quantitative studies also show that in those cases where the female dominates, a very great volumetric preponderance of the female gonads is usually seen, and is probably necessary. This preponderance is visible early in development, but it is not yet clear how it becomes established.

The process of transformation from female to male in the pairs of this series has recently been described (Burns, '30).

18. *Studies on amphibian endocrines. IV. The reaction of elements of the pars anterior of the pituitary gland of Necturus maculosus to vital dyes and to some accepted mitochondrial and Golgi techniques.* HARRY A. CHARIPPER (introduced by Alden B. Dawson), New York University.

This is the second of a series of studies undertaken to determine if there are any differences in the histological picture obtained by usual fixation methods as compared with the vital techniques now in vogue. A study of sections of the pars anterior of the pituitary gland of Necturus maculosus subjected to the Nassonov-Kolatchev, Champy-Kull, and Mann-Kopsch methods shows the presence of osmiophilic substance (rings and granules), granular mitochondria, and secretory granules. The secretory granules can also be demonstrated in material stained with neutral-gentian following a Zenker-formic-osmic fixation. Fresh anterior pituitary removed from animals of approximately the same age as those used for the fixed material was exposed to neutral red and Janus green B. In tissue exposed to Janus green B granular cytoplasmic inclusions appear in a few seconds. These elements show approximately the same distribution as the mitochondria obtained by the methods of Mann-Kopsch and Champy-Kull. Cells exposed to neutral red alone for three to five minutes show large and small granules. These appear in the same general area as do the osmiophilic substances and secretory granules. There is no apparent radical difference between the picture of cytoplasmic inclusions obtained by the usual fixation and staining methods and that obtained by vital staining.

19. *Studies on amphibian endocrines. V. Accelerated metamorphosis of Rana clamitans larvae by means of implants of fresh anterior pituitary from adult Necturus maculosus.* HARRY A. CHARIPPER AND EDWARD L. COREY (introduced by F. Gudernatsch), New York University and University of Virginia.

Implants of thyroid tissue from Necturus maculosus, a neotenic form, have been shown to bring about accelerated metamorphosis in frog larvae (Swingle). Since the control of amphibian metamorphosis is known to be dependent upon the interrelated activity of the thyroid and pituitary glands, it seemed advisable to test the anterior pituitary of Necturus in regard to its potentialities for producing metamorphosis. Accordingly, larvae of Rana clamitans were narcotized in a dilute solution of chloretone and implanted with a piece of the pars anterior obtained from adult Necturi. The implants varied in size from one-half to the entire portion of the gland. After six weeks, the experimental animals showed well-developed hind limbs, the left fore limb was visible, the tail was considerably atrophied, and the intestinal tract gave definite evidence of metamorphic changes. The control animals exhibited no changes characteristic of metamorphosis. The larvae receiving the larger implants were further advanced in respect to the development of their hind limbs and the extent of atrophy of the tail. An attempt is being made to carry some of the animals through to complete metamorphosis.

20. *Innervation of the pia mater of the spinal cord.* Sam L. Clark, Northwestern University Medical School.

With the pyridin-silver method, the nerve fibers and endings were stained in the spinal pia mater of adult cats. After staining and dehydration, the pia mater was stripped from segments of the cord and mounted intact on slides for microscopic study. The spinal pia mater in the cat is supplied with numerous bundles of nerve fibers, the majority of which extend lengthwise the cord. These bundles contain few myelinated and many unmyelinated fibers, the latter being derived in part from the former. Exclusive of the 'vasomotor' nerves to the arterial system, the nerve fibers end on the pia itself, and on or near blood vessels in terminations of 'sensory' type. Nerve fibers enter the spinal pia mater from the white matter of the cord, the dorsal roots, and the ventral roots. It is not possible from the microscopic appearance to draw conclusions as to the function of the nerve fibers and endings in the spinal pia mater. It appears that their arrangement and distribution are in part dependent on the mechanical influences of the terrain on developing neural elements.

21. *The development of the brain of Bdellostoma stouti. II. Internal growth changes.* J. LeRoy Conel, Department of Anatomy, Boston University.

Practically all the parts which have been described in the brains of lower vertebrates are present in the brain of Cyclostomes, but the usual relations have been so disturbed by external pressure that identification of these parts in the adult brain is difficult. They can be identified in young embryos, however, and can be followed to the definitive condition through the successive growth changes during development.

The brain of a young embryo which had been relieved of external pressure by rupture of the shell resembles the embryonic brain of Necturus.

In the early stages of development the primordium hippocampi is external in position, but it is pushed to a central position by adjacent parts in the late stages.

A laminated cortex can be distinguished in the hemisphere.

The ventricular system is at first large and complete, but in older embryos it becomes greatly reduced, and in many places is entirely obliterated.

22. *Studies on the leucocyte count of pregnant rats.* Arthur M. Crosman (introduced by F. Gudernatsch), Washington Square College, New York University.

Investigations on the problem of the absorption of dead fetuses in the rat indicated that during absorption the leucocyte count was affected. It, therefore, became necessary to determine the leucocyte picture of normal pregnant rats so as to have a standard for comparison.

The present data show: 1) The average count of a pregnant rat is approximately 7000 to 10,000 higher than that of a non-pregnant one. 2) There is an increase of from 3000 to 12,000 cells per cubic millimeter on the first day of pregnancy. 3) Between the seventh and eleventh days of gestation, there is a sudden marked increase of short duration and then a return to the previous level. 4) At the time of parturition a marked decrease occurs. This decrease may start three or four days before delivery. Its lowest point, which may be reached shortly before, during, or just after parturition, generally lies below the level observed in a normal non-pregnant rat. Work is still in progress to complete the above data.

23. *Changes in the erythrocytes of Necturus associated with the intracellular crystallization of hemoglobin.* ALDEN B. DAWSON, Zoölogical Laboratory, Harvard University.

Intracellular crystallization of hemoglobin may be readily induced, in the erythrocytes of animals previously poisoned by prolonged immersion in lead acetate, by the momentary drying of drops of blood on the slide before covering. Only the peripheral cells in contact with the glass are so affected, and they may accordingly be found distributed in a circular pattern in the covered preparation. All or part of the hemoglobin within a cell may become crystallized. The number of crystals formed in each cell varies, one, two, and three being the most common. Even in cells not stained supravitally, the slender filamentous mitochondria may become visible as the cytoplasm clears during the formation of a crystal. In supravitally stained preparations the neutral-red bodies and mitochondria are more easily observed and in some cases a few of these elements may become incorporated within a crystal. Crystal formation is associated with a greatly decreased viscosity of the cell contents, the cell membrane becomes conspicuous, and in many instances the crystals become large enough to cause great distortion of the cell. High concentrations of neutral red do not induce reticulation in these modified cells.

24. *A study of hair growth in the guinea-pig (Cavia cobaya).* HELEN L. DAWSON (introduced by Mildred Trotter), Department of Anatomy, Washington University, St. Louis.

Five distinct types of hairs, differentiated on the basis of size and designated as types 1, 2, 3, 4, and 5, were found in the coat of the guinea-pig in the regions of the hip, shoulder, and abdomen. A study of the hair in these regions of fifteen mature white guinea-pigs presented the following results.

Types 1 and 2 predominated (about 75 per cent), type 5 occurred rarely (less than 1 per cent), and types 3 and 4 constituted the remainder. The hairs were arranged in linear groups and each group consisted of from five to fourteen hairs. The groups containing eight hairs predominated in all three regions. The length of the period of growth averaged four weeks, during which time the rate of growth of the hairs varied from 2 to 5 mm. weekly, depending upon the ultimate length of the hair and the length of the period of growth. Pregnancy prolonged the period of rest and consequently the length of the cycle. The majority of the hairs remained quiescent in the follicle for a period of twelve weeks in males and virgin females and fifteen weeks in females which had been pregnant. No correlations were found between the types of hairs and periods of growth and of rest.

25. Hair groupings on the venter of the adult albino rat. Doris A. Fraser (introduced by W. H. F. Addison), Department of Anatomy, University of Pennsylvania.

Sections of skin at right angles to the length of hair follicles were studied. Follicles appeared in a somewhat linear form arranged transversely on the body. This linear arrangement could be divided into more or less definitely delimited groups. These groups were of two types: those with, and those without, a larger central follicle. Over a period of four winter months the average number of groups per square millimeter of ventral skin was 11.5. Of these, 5.3 groups were arranged with a central follicle, and 6.2 groups without. In a group with a central follicle, the number of additional follicles varied from three to eight, and the average number of hairs to a follicle was 2.3. The central follicle usually contained only one hair. Total hairs in such a group averaged 12.7. Groups without a central follicle were mostly of three or four follicles averaging 2.7 hairs to a follicle. The total hairs in such a group averaged 8.8. The total number of follicles in 1 sq.mm. of ventral skin averaged 54.7. The average number of hairs per square millimeter of ventral skin was 152.6. Compared with the average of the dorsal lumbar regions of the same animals, there were four more groups, nineteen more follicles, and sixty-seven more hairs to the square millimeter on the venter.

26. Metamorphosis of the axolotl following lung extirpation. S. T. Garber (introduced by W. W. Swingle), Biological Laboratory, Princeton University.

Various theories have been advanced to account for the non-metamorphosis of certain perennibranchiate amphibians such as Necturus.

The most recent of these theories is that advanced by Figge ('29). To test his theory, "Namely, that the sixth aortic arch is necessary for metamorphosis and that this is absent in Necturus," Figge ligated the arch and vessels on each side in axolotls—a metamorphosing form—and found that the animals did not transform when fed metamorphosing substances.

In view of the fact that certain lungless salamanders do metamorphose, it was considered unlikely that pulmonary respiration should play such an important rôle in the metamorphosis of axolotls. Consequently, the experiment was undertaken of attempting to metamorphose axolotls following lung extirpation. The lungs were removed from a series of axolotls, and it was found on feeding the operated larvae with desiccated thyroid glands, that they metamorphosed in exactly the same manner as the non-operated animals when similarly fed. Autopsy of the operated axolotls showed that the lung had been completely removed. Since Figge's experiment of ligating the sixth arch is essentially the same, in that it cuts off pulmonary respiration, his conclusion is evidently erroneous that "the sixth arch is necessary for metamorphosis," since axolotls have here been made to metamorphose completely without lungs.

27. *The relation of decerebrate rigidity to the level of transection of the brain stem in young rabbits.* **Albert M. Griffin** (introduced by William F. Windle), Northwestern University Medical School.

In a series of rabbits one to eight days old the brain stem was transected at three principal levels, producing 'hypothalamic,' 'midbrain,' and 'pontine' preparations.

In hypothalamic animals the section passed from the superior colliculi to the optic chiasma. Prolonged responses to slight stimuli, spontaneous running movements lasting as long as thirty minutes, and persistence of righting reflexes characterized them.

Midbrain animals, in which the section passed from the superior colliculi to the anterior border of the pons, exhibited typical decerebrate rigidity, oftentimes so marked that the animals could support their weight upon their legs. Rigidity developed shortly after the operation and persisted one to three hours.

Several animals, prepared by passing the knife from the inferior colliculi to the middle of the pons, developed flexor rigidity. The entire body of these was in a state of hyperflexion; the vertebral column was arched dorsally, and fore and hind legs exhibited adduction and flexion which resisted passive extension. Spontaneous movements were feeble and purposeless, and the production of distinct reflex responses required stronger stimuli than were used in the other animals. The exact nature of the lesions in this group is undetermined, but eighth-nerve involvement is suspected.

28. *Roentgenological studies on the gall bladder of the dog. Experiments with lipiodol.* **Béla Halpert and Allan G. Rewbridge**, Department of Pathology and the Otho S. A. Sprague Memorial Institute, University of Chicago.

Normal healthy dogs were laparotomized and 2 cc. of lipiodol introduced into their gall bladders after 3 cc. of bile had been withdrawn. The animals were replaced in their usual environment and kept on their usual diet. Roentgenograms were taken six hours after operation and subsequently once daily until the shadow had vanished or remained stationary for several days. In the latter case, a meal, consisting of chopped meat and two eggs, was fed, and a roentgenogram taken three hours later and one the following day.

The shadows on the first films averaged about 4 × 2 cm. and imitated the shape of the viscus. Of the twenty animals used, the shadow disappeared completely in one the first day, in seven between the second and fourth, in six between the fifth and tenth days, and in one on the seventeenth day. In the remaining five dogs the shadow diminished to about 25 per cent of its original size and remained unaltered until the experiment was concluded from sixteen to twenty-six days after operation. Thus lipiodol was completely eliminated from the gall bladder in 75 per cent of the dogs, whereas complete evacuation did not occur in 25 per cent. According to the above observations, complete emptying of the dog's gall bladder does not occur with each meal.

29. *Morphological studies on the gall bladder and the biliary passages of the rabbit.* Béla Halpert and Gertrude H. Wilber, Department of Pathology and the Otho S. A. Sprague Memorial Institute, University of Chicago.

The capacity of the rabbit's gall bladder averages 1 to 2 cc. per kilogram of body weight. A definite fossa vesicae felleae surrounds about two-thirds to four-fifths of the distended gall bladder. There is an S-shaped curve about the junction of the neck and cystic duct. The orifice of the latter is eccentrically situated; Heisterian folds have not been observed. The cystic duct branches off from a hepatic duct. The muscular coat of the duodenum is increased in thickness where the ductus choledochus merges into it.

The surface pattern of the gall-bladder mucosa consists of a network of delicate folds of approximately the same width and height. The latter is about three times that of the entire thickness of the rest of the wall. *a*) The lining epithelium is tall columnar and rests upon a thin tunica propria mucosae. The cells have no cuticula. Rokitansky-Aschoff sinuses are frequent. *b*) Most of the bundles of the muscular coat are longitudinal or oblique. They are scant or absent on the side attached to the liver. *c*) The perimuscular coat contains the middle-sized vessels. *d*) The serosa covers four-fifths of the entire surface of the viscus.

30. *Bimanual rectal palpation as applied to the female rhesus monkey.* Carl G. Hartman, Department of Embryology, Carnegie Institution of Washington, Baltimore.

With the middle finger of one hand in the rectum, the free hand on the abdomen, it is possible to palpate the uterus and both ovaries of the female monkey with infinitely greater ease and definiteness than in human patients. It is possible to follow the changes in the uterus throughout the menstrual cycle, both as to size and consistency. A premenstrual enlargement and slight softening indicate an ovulatory cycle—a conclusion that can be checked by the changing size and shape differences between the ovaries. Laparotomy is not necessary to distinguish ovulatory and non-ovulatory cycles. Pregnancy changes are most interesting. The initial softening involves the entire uterus, at first like a well-developed premenstrual organ, then progressively softer. Later, the lower segment and cervix, while the fundus with its ovum is still fairly firm, become so yielding to the touch as almost to pass out of the picture. Midway through pregnancy or later the vagina begins to enlarge and soften also. The details are now being worked out on twelve pregnant and several dozen non-pregnant animals.

31. *Caudal proportions of ricocheting Rodentia.* Robert T. Hatt (introduced by G. K. Noble), The American Museum of Natural History.

Five divergent groups of Rodentia have independently developed ricochetory types. All have developed long tails for counterpoise. It is demonstrated that the tail length is not directly proportional to degree of ricochetory specialization, but varies with the relative massiveness of the tail and the degree of terminal hairing. Jaculus lichtensteini appears to be the most highly specialized for this type of progression as indicated by the ratio of the foot to body length, 65 per cent. The tail is 159 per cent of the body length and bears a strong terminal tuft, which through added wind resistance compensates for lack of greater length. Zapus pacificus, an animal of about the same size, has a foot ratio of only 37 per cent, but a caudal ratio of 168 per cent. This tail, which is proportionately the longest of all, is not tufted. Pedetes surdaster, a much larger animal, has a foot ratio of 43 per cent and a tuftless tail of only 122 per cent. The tail is, however, heavy, in contrast to the two former extremes. Intermediately specialized forms demonstrate that tail length and tufting are characters not dependent directly on the body weight to be counterbalanced, but on the degree of ricochetory development.

32. *Transformation of the modified ('freemartin') ovary of Amblystoma tigrinum into a functional testis.* R. R. HUMPHREY, University of Buffalo.

The ovary of Amblystoma tigrinum may be reduced to a rudimentary or vestigial state under the influence of a testis present in the same host (Humphrey, '29) or the influence of a male joined in parabiosis to a female (Burns, '30). The central cavity of the ovary is suppressed; the growth of the ovarian cortex is inhibited and its oocytes undergo degeneration; the cortex eventually is reduced to a germinal epithelium containing a few primary oogonia. The proximal (hilar or medullary) portion of the gonad becomes relatively prominent; where germ cells in small numbers are associated with its rete cords, it exhibits the appearance of a rudimentary testis. The modified ovary may remain in this state more or less indefinitely, or may transform in a part of its extent into a testis of normal structure. The prerequisite for such transformation is the presence along some portion of the ovary of a considerable number of germ cells located at the hilus or in the mesovarium near it; these proliferate and produce testicular lobules, converting a portion of the ovary into a functional testis, while the remainder of the gonad persists in the rudimentary state. Dissections and microscopic preparations will be demonstrated, showing the modified ovary and various stages in its transformation into a testis, including advanced stages in which mature spermatoza have been produced.

33. *Studies on ovulation induced by extracts of the hypophysis and by the urine of pregnant women.* JOHN J. JARES, JR. (introduced by G. W. Corner), Department of Anatomy, University of Rochester.

Efforts to induce ovulation in rabbits by the recent method of Bellerby failed, although extracts of the anterior lobe made similarly, but omitting the kaolin treatment and substituting centrifugalization for filtration, induced ovulation. An alkaline extract, prepared by Dr. E. P. Bugbee, of Parke, Davis & Co., also induced ovulation by a single intravenous dose; the potent substance does not dialyze from the extract. A single intravenous injection of the untreated urine of a pregnant woman will induce ovulation (confirming M. H. Friedman), in a dose of about 0.3 cc. or more, ten hours after injection. Larger doses do not hasten the effect. The presence of corpora lutea does not inhibit the ovulatory effect, and rabbits may be kept constantly provided with corpora lutea by intravenous injections of urine from pregnant women at ten-day intervals.

34. *The influence of light and temperature on the retinal elements of the excised eye of the frog.* W. K. Jennings (introduced by L. B. Arey), Northwestern University Medical School.

The excised eyes of a light-adapted frog, when placed in its own peritoneal cavity or in that of another frog at room temperature, will respond to darkness by a variable retraction of the retinal pigment and an elongation of the cones. This is in agreement with Kumagai's ('16) unsupported assertion and indicates that the peritoneal fluid is a more favorable medium than the common physiological solutions, in which this particular type of response fails (Arey, '16).

Experiments with temperature on eyes retained in the peritoneal cavity show that low temperature has little or no influence in changing the distribution of pigment and the positions of rods and cones over that maintained at an intermediate room condition. High temperature, on the contrary, causes a retraction of pigment and an elongation of rods; an effect on cones, if present, is slight. The rod response is without precedent in accounts hitherto reported.

35. *Effect of lecithin on the sex ratio in the albino rat.* H. E. Jordan and W. H. Paine, University of Virginia.

On the basis of results with rabbits, Russo ('09) claimed that the administration of lecithin changed the sex ratio in favor of females. In ten control litters the males totaled thirty-six; the females, twenty-nine. The ten experimental litters gave a proportion of twenty-six males to forty females. Following Russo's technique, we obtained similar results with the white rat. Seventy-five control litters gave a proportion of 375 males to 361 females. In one series of thirteen litters injected with lecithin we obtained a proportion of forty-nine males to seventy-three females. In another series in which a first control litter was followed by an experimental litter from the same females, the proportion of the total of twenty-five litters was as follows: control litters, 121 males to 102 females; litters after lecithin injection, eighty-eight males to 107 females. While our results are like those of Russo, the significance of both with reference to a sex-determining influence of lecithin seems dubious by reason of the fact that both in the control and experimental series the individual litters varied greatly, with preponderance, respectively, in favor of both males and females. Moreover, while most groups of ten consecutive litters in the first control series of seventy-five litters show a preponderance of males, a few groups may be selected in which females preponderate.

36. *The reticulo-fibrillar apparatus of red blood corpuscles in relation to Cabot rings.* H. E. Jordan, J. E. Kindred, and H. W. Beams, University of Virginia.

Golgi ('20) described a unique central network in the red corpuscles of man and normoblasts of the guinea-pig. In anucleate biconcave discs it bounds the central depression; in erythrocytes it surrounds the nucleus. It is said to correspond to no previously described cell structure. Rings of Cabot ('03), found in the red corpuscles in severe anemias, have been quite generally interpreted as nuclear remnants. Cesaris-Demel ('21) seeks to identify Cabot rings with the boundary of the newly discovered 'reticulo-fibrillar apparatus' of Golgi. We have studied Cabot rings in the red corpuscles from a case of von Jaksch's anemia. The evidence indicates origin from a persistent nuclear membrane. In some cases the ring is extruded after complete disapparance of the intranuclear substance. The reticulo-fibrillar apparatus is readily demonstrable in red corpuscles of mammals and red cells of amphibians. After laking, the apparatus is dispersed throughout the cytoplasm. Appropriate techniques reveal in the immediate perinuclear area: pigment (salamander), concentration of hemoglobin (Myxine), mitochondria (Necturus). No evidence accrues that Cabot rings arise from any of the perinuclear substances. The fact that in cells where nuclear resorption is preceded by fragmentation the nuclear partition products form smaller rings supports the interpretation of Cabot bodies as persistent nuclear membranes. It seems probable that the reticulo-fibrillar apparatus of Golgi may represent a denser or more robust perinuclear stroma.

37. The anatomy of the female pelvis: a cinematographic presentation with sound. Howard B. Kellogg and William F. Windle, Northwestern University Medical School.

The main purpose of the present film is to furnish a brief review of the anatomical structures of importance to clinical men. Effective presentation of this subject is enhanced by a brief description synchronized with the picture.

38. The brain of the porpoise (Tursiops truncatus). Orthello R. Langworthy, The Johns Hopkins University.

The brain of the porpoise (Tursiops truncatus) is foreshortened in its anteroposterior extent and widened laterally, due to changes in the conformation of the skull. The cerebral hemispheres are large and show a great number of convolutions and sulci. In this respect they are more highly differentiated than the cerebral hemispheres of man. The cerebellum and particularly the cerebellar hemispheres are large; the flocculus is well developed. A massive tract of fibers connects the cerebral and cerebellar hemispheres. No olfactory nerve fibers or olfactory bulb are present in the porpoise and the secondary olfactory end stations in the brain are rudimentary. The cochlear nerve and its connections with the nervous system are strongly developed.

Microscopic study of the gray matter of the cerebral cortex demonstrated that it has a very primitive structure, with few cells and poor differentiation of the cell layers. A large motor area and an area frontalis are present at the extreme frontal pole of the cortex. The motor cortex and the projection areas appear to be surrounded by large areas of undifferentiated cortex. The granular layer is recognizable everywhere except in the motor cortex. The supragranular pyramidal layer is thin throughout the cortex.

39. The quantitative distribution of the pyramidal tract in the dog. Arthur M. Lassek, Leslie W. Dowd, and Arthur Weil (introduced by L. B. Arey). Department of Anatomy and Institute of Neurology, Northwestern University Medical School.

In studying degenerated pyramidal tracts stained by the Marchi, Pal-Weigert, and Weil methods, it was calculated that 55 per cent of the tract in the spinal cord of the dog passes to the cervical segments, 20 per cent goes to the thoracic segments, and 25 per cent is distributed to the lumbar and sacral segments. The cervical segments supply 40 per cent of the musculature, the thoracic segments 25 per cent, and the lumbar and sacral segments 35 per cent. The number of pyramidal-tract fibers which supply the different groups of spinal-cord segments was estimated, and from this gross number was computed the amount for 1 gram of muscle: C1–4, 104 fibers; C5–T2, 24; T3–13, 21; and L1–S3, 21 fibers to every gram. In a previous investigation on man, Weil and Lassek ('29) found that the relative distribution of pyramidal-tract fibers within the spinal cord is the same as in the dog. The number of nerve fibers corresponding to 1 gram of muscle is the same in the cervical and lumbar segments of man and dog. The discrepancy in the supply of musculature innervated by T3–11 in man (sixty-two and twenty-one fibers, respectively) is explained by the fact that the weight of the muscles in the thoracic region of man is only 6.8 per cent of the total musculature which is innervated by the spinal cord, as compared with 24.2 per cent in the dog.

40. Prenatal growth in weight of the head, trunk, fore limbs, and hind limbs of the cat. HOMER B. LATIMER, University of Kansas.

A series of 264 cat embryos weighing from 0.3 to 197 grams was studied. This group includes thirty-five newborns ranging from 97 to 197 grams in weight.

The curve representing the weight of the trunk plotted against body weight is slightly concave superiorly, while the corresponding curves for the head, fore limbs, and hind limbs are slightly convex superiorly.

The weights of these four divisions plotted against nose-anus length form curves concave superiorly, rising slowly at first and more rapidly toward the end.

The weight of each part was reduced to a percentage of the total body weight and plotted against body weight. The curve representing the head percentage falls rapidly during the first 30 grams' increase in body weight and then more slowly. The percentage curves for the trunk, fore limbs, and hind limbs all rise rapidly at first. The percentage of the trunk continues to rise more slowly throughout the growth period. The percentage curve of the fore limbs rises rapidly to its maximum of about 8 per cent at 30 grams of body weight, then slowly decreases to a little less than 7 per cent. The maximum of 10.5 per cent for the hind limbs is reached at 80 grams of body weight and it likewise decreases slightly thereafter.

There is no apparent sex difference in either the weights or the percentages plotted against body weight or body length.

41. Effects of repeated short exposures to x-ray irradiation on the blood and hemopoietic organs. JOHN STEPHENS LATTA, Department of Anatomy, College of Medicine, University of Nebraska.

A series of albino rats were subjected to short exposures to x-rays totalling four hours, with blood examinations during the course of experiments. Conditions existing in the hemopoietic organs after total exposures were determined and an attempt made to correlate these with the changed blood picture.

The total white counts were reduced immediately, reaching 1000 cells per cubic millimeter after two hours' irradiation, and sometimes reaching 500 after four hours. The average of differential counts showed a complete reversal of percentages of neutrophiles and lymphocytes; before irradiation: neutrophiles, 20 per cent; lymphocytes, 70 per cent; after irradiation: neutrophiles, 73 per cent; lymphocytes, 19 per cent. The first definite decrease in erythrocytes occurred after forty-five minutes' exposure; after three hours, polychromatophilia, anisocytosis, and normoblasts were present. After four hours, the average reduction in erythrocytes was nearly six million.

Tissue examination showed almost complete disappearance of lymphocytes from the spleen, and marked reduction in the lymphatic nodes coinciding with the tremendous actual and relative lymphopenia in the blood. The spleen and nodes were heavily infiltrated with neutrophiles, indicating withdrawal from the circulation. This leucopenia was further explained by a great decrease in the granuloblasts and hemoblasts (myeloblasts?) of the marrow. The anemia was apparently due to a developmental factor as indicated by abnormalities of erythrocytes in the blood and marked evidences of degeneration in megablastic foci coupled with reduction in hemoblasts. There was complete aplasia of megakaryocytes.

42. *The effect of oestrin on the growth of the ovary in immature female rats.* **Samuel L. Leonard, R. K. Meyer, and Frederick L. Hisaw** (introduced by M. F. Guyer), Zoölogy Department, University of Wisconsin.

In immature female rats twenty to twenty-five days old given daily injections of 2 to 4 rat units of oil-soluble oestrin for twenty to sixty-seven consecutive days ovarian development is markedly influenced. It was found that the weight of the ovaries of rats receiving oestrin was, on an average, 33 per cent lighter than that of their litter-mate controls injected with an equivalent amount of oil minus the hormone. These results are positive only when the injections are continued beyond the time of normal sexual maturity. Sexual maturity occurred in a few of the experimental animals, as indicated by the presence of corpora lutea, but in such the number of follicles developed was significantly fewer than in the controls. This seems to show that oestrin in the doses given does not completely inhibit ovulation.

When the pituitaries of the control and oestrin-treated litter-mate females were transplanted into immature female rats, the pituitaries of the non-treated animals were found to be more potent in the gonad stimulator; this was determined by the fact that the vaginas of immature rats receiving the experimental pituitary opened from three to fifteen days after the controls. These results seem to indicate that the secretion of the gonad stimulator is in some way influenced by the administration of oestrin.

43. *Nuclear structures of malignant cells.* **Margaret Reed Lewis**, Department of Embryology, Carnegie Institution of Washington, Baltimore.

The material studied included tissue cultures and sections of chicken sarcoma, rabbit myxoma, mouse sarcoma, three rat sarcomas, three rat carcinomas, and several human tumors.

All malignant cells exhibited some hypertrophy of chromatin and of nucleolar material. Some tumors, especially no. 256, had malignant cells characterized by such greatly hypertrophied plasmosomes that they resembled the herpetic nuclear inclusion bodies both in size and staining reaction. Other tumors (Walker 1 and 155) had a greatly increased number and size of karyosomes as well as some hypertrophy of the plasmosomes. Two tumors (Crocker 10 and Walker 135) had malignant cells with only slightly increased chromatin and nucleolar material.

The malignant cell of each tumor exhibited characteristic nuclear structures, and these did not become changed through many generations of transplantations in vivo.

44. *The chromosomes of malignant cells.* MARGARET REED LEWIS, Deparment of Embryology, Carnegie Institution of Washington, Baltimore.

Observations on tissue cultures and sections of ten types of tumors showed that tumors had many normal as well as malignant cells undergoing mitosis. The normal cells in all tumors exhibited the normal number of chromosomes, while the malignant cells of some tumors had a greater number than normal. An occasional abnormal mitotic figure was seen.

The malignant cells of Crocker 10, Walker 135 and 20 exhibited the normal number (forty-two), while the malignant cells of the Walker 1 and 155 had the tetraploid number (eighty-four) of chromosomes. The Walker carcinoma 256 had a hypertrophied number and the Walker carcinoma 76 had some epithelial cells with the normal number and some with a greatly increased number of chromosomes. Some human tumors had malignant cells with the normal number, and other human tumors had malignant cells with more chromosomes than normal. One metastatic growth from a breast carcinoma had malignant cells which exhibited a tetraploid number of chromosomes.

The number of chromosomes characteristic of a given tumor remained unchanged through many transplantations in vivo.

45. *Indications of secretory activity of the glomerular epithelium of the rat kidney.* WARREN H. LEWIS, Department of Embryology, Carnegie Institution of Washington, Baltimore.

Within three hours after small pieces (1 mm. diameter) of adult rat kidney are immersed in Locke solution, large amounts of fluid often collect within the glomerular capsules between Bowman's membrane and the capillary tuft, pushing the latter to one side and distending the capsule. Various other tissues treated in the same manner give no indication of the collection of fluid, hence we conclude that the glomerular epithelium over the capillaries and perhaps also the outer layer probably play an active rôle in the secretion of fluid, rather than merely allowing fluid to passively filter through the cells under vascular pressure. We assume that the convoluted tubules were obstructed by the manipulation of the swollen glomeruli, while the tubules of the glomeruli which did not collect fluid remained open. The epithelial as well as various other types of cells remain alive for many hours after removal from the body.

46. *External and internal anatomy of the forebrain of the opossum.* Y. T. Loo, (introduced by C. Judson Herrick), University of Chicago.

Drawings, specimens, and models illustrating surface features and gross internal structure. The dissections were made under a low-power microscope after an exhaustive study of microscopic structure and in the light of knowledge so acquired. Despite the small size of this primitive brain, some very complicated fiber systems, like the internal capsule, can be analyzed by this method more completely than is possible in higher mammalian brains, where the primary systems of fibers cannot be so clearly separated.

47. *A histological study of the thymus gland in Alligator mississippiensis.* J. B. Looper, University of Mississippi School of Medicine.

The thymus gland in the alligator consists of two elongated parts lying on either side of the neck in close proximity to the bifurcation of the carotid arteries. Parts of the parathyroid glands are close by, and in some cases enclosed in the same connective-tissue capsule. Each elongated part of the thymus is composed of three or more lobules which are frequently separated from each other by considerable connective tissue. Delicate septa from the enveloping capsule enter the parenchyma. The collagenous fibers of the trabeculae are continuous with the argyrophile fibers of the reticular stroma. There is no distinct demarcation between the cortical and medullary regions as found in the thymus of mammals. The cellular elements are more or less uniformly distributed, and consist chiefly of lymphocytes, monocytes, and reticulum cells. However, in smaller numbers are found eosinophilic granulocytes, basophilic granulocytes, amphophilic granulocytes, Russell-body cells, and cells which resemble plasma cells. The granulocytes are found largely just beneath the capsule and along the trabeculae.

In addition to the tissues and cells mentioned above, the thymus of the alligator contains unicellular concentric corpuscles of Hassall, various syncytial masses, and cystic formations. All of these apparently arise from the entodermal epithelium enclosed in the early formation of the thymus.

The thymus glands of old alligators show marked general involution.

48. *Biological researches with the ultraviolet microscope illustrating recent developments by optical sectioning.* Francis F. Lucas and Mary B. Stark, Bell Telephone Laboratories and Flower Hospital, New York City.

By means of this new development, a transparent specimen such as a group of cells may be sectioned optically. The focal planes may be spaced as closely as $\frac{1}{16}\,\mu$. Successive photographs give a progressive record of the structure. Magnifications as high as 5000 diameters result in crisp brilliant images with a degree of resolution surpassing by far that achieved with any other optical system. The application of this method has been made to the study of living cells of the following: white-mouse tumors; spermatogenesis of the grasshopper; spermatogenesis of the white mouse; life history of Hymenolepis murina; various regions of the brain of the white mouse and guinea-pig.

Detailed directions regarding this method will shortly appear in a scientific publication.

49. *Factors affecting the rhythmic variations in the vascularity of the uterus of the rabbit.* J. E. MARKEE, Department of Anatomy, Stanford University.

The vascularity of uterine transplants upon the rabbit's iris undergoes rhythmic variations similar to those described by me in the guinea-pig. In quiet rabbits vasoconstriction in the transplant occurs about every seventy seconds. The periodicity of these changes is modified by fright. For convenience, each vascular cycle may be divided into four parts: vasoconstriction, ten seconds; refractory period, fifteen seconds; resting period, twenty-five seconds, and latent period, twenty seconds. If a rabbit is frightened when the transplant is in the resting phase of a vascular cycle, that cycle is shortened. A vasoconstriction occurs only in this phase, twenty seconds after fright. Fright produced in any other phase does not shorten the cycle.

The fact that two transplants in the same eye have different rhythms in undisturbed rabbits seems to indicate that in the resting condition the length of the rhythm is determined by some factor within the transplants. However, when a rabbit is frightened, vasoconstriction occurs simultaneously in adjacent transplants if both are in the resting phase. It seems, therefore, that the control of the vasoconstriction due to fright must be located outside the transplants. Removal of neither the superior cervical ganglion nor the ciliary ganglion affects these vasoconstrictions. After double adrenalectomy only the rhythmic vascular changes of the spontaneous type occur.

50. *The effects of vitamin-E administration on the testis in partial inanition and vitamin-D deficiency.* KARL E. MASON, Department of Anatomy, Vanderbilt University School of Medicine.

It has been shown by Mattill ('27) and Evans ('28) that rats can maintain testis normality under conditions of severe vitamin-B deficiency, providing sufficient quantities of vitamin E are supplied. In vitamin-A deficiency testicular changes result despite vitamin-E therapy (Mason, '30). More recent studies have dealt with the efficacy of E therapy on the testes of rats subjected to varying degrees of inanition and to vitamin-D deficiency. Daily administration of ten and twenty drops of wheat-germ oil has failed to give any evident stimulation of maturation or protection against degenerative changes in rats maintained at a constant body weight of 40 grams for approximately thirty-day periods. Neither was any protection afforded in sexually mature rats of 100 to 200 grams body weight reduced gradually to, and maintained at, approximately one-half their original weight for various periods. Both graham bread plus raw milk and purified food diets, supplemented with more than adequate amounts of all known vitamins, were used. Studies in rats maintained on various types of rachitogenic diets, with and without supplements of ten and twenty drops of wheat-germ oil, have given results similar to the above series. Whether the vitamin or salt factors are the more important in the latter type of experiment is under investigation.

51. *High-frequency equipment for biology.* G. MURRAY MCKINLEY (introduced by H. H. Collins), University of Pittsburgh.

Growing interest in biological effects of very high-frequency oscillations led to design of convenient apparatus for treatment of small laboratory animals. Apparatus has wave-length range of 2 to 6 meters, with all parts mounted on wooden panel.

Auxiliary current is of sufficient strength to kill large rats in ten minutes. Change of frequency and tuning is done with sliding shunts; easy interchange of condenser plates is provided for. Transformers, rheostat, vacuum tube, chokes, resistances, etc., are all standard radio equipment; 110 volt, A.C., is used. UV 203, 50-watt vacuum tube is used and experience has given best possible circuit for treatment of laboratory material.

All the parts are standard radio equipment and are of low cost.

52. Cell groups in the tuber cinereum of the dog. **Lawrence O. Morgan**, University of Cincinnati College of Medicine.

The cell groups in the tuber cinereum of the dog most closely resemble those described by Malone in the human brain (substantia grisea, nucleus tuberis lateralis, and nucleus tuberomamillaris).

There is a substantia grisea composed of small cells with scant cytoplasm. They are most concentrated in the immediate wall of the third ventricle, from the optic chiasma to the mammillary body, and thin out laterally in the region of the fornix. The nucleus tuberis lateralis is composed of slightly larger cells, elongated in form, with a relatively large amount of darkly staining cytoplasm. This nucleus is not well circumscribed as in the human. The cells are most concentrated along the base of the tuber and thin out in the region of the fornix. The nucleus tuberomamillaris consists of large cells containing a large amount of darkly staining chromatin. They are collected around the fornix and scatter into the regions dorsal and ventral to the fornix. The three cell groups intermingle freely in the region of the fornix.

Considerable evidence has been produced indicating that these cell groups in the tuber are secretory centers for certain endocrine glands, especially the thyroids (nucleus tuberomamillaris?) and suprarenals (substantia grisea?). The author has described marked pathological changes in these nuclei in human epilepsy and produced epileptiform convulsions by placing lesions in the tuber of the dog.

53. An experimental study of the ovarian blood supply in the rat. **W. O. Nelson** (introduced by J. F. Gudernatsch), Department of Biology, New York University.

Two series of animals were used, the first consisting of twenty-four nonpregnant rats whose estrous cycles had been found normal for several weeks prior to and following unilateral ovariectomy. The ovarian and the upper end of the uterine arteries were ligated (single or double ligation with resection). In some cases the cycle resumed its normal rhythm after a few days, in others after a longer period, and in the remaining instances it was not resumed up to the time of autopsy. All animals were killed and their arteries injected. Animals resuming a normal cycle displayed either the development of a collateral supply or a reunion of the vessel about the ligature so that an adequate supply was reestablished. When the cycle was stopped, no arterial supply could be demonstrated. Several animals were simply unilaterally ligated with no resultant effect on the cycle.

In the second series (eighteen rats) unilateral ligation was employed, as in the first series, either shortly before or after conception. The remaining ovary was extirpated about the thirteenth day of gestation. Some animals went to term, the others either aborted or resorbed their foetuses. Postmortem injection demonstrated a renewed arterial supply in the former animals and its absence in the latter. Control ligations of the uterine artery, near the tube, had no effect upon pregnancy.

54. *The effect of alcohol upon the estrous cycle of the rat.* W. O. Nelson (introduced by J. F. Gudernatsch), Department of Biology, New York University.

Two series of female rats were placed daily in a chamber saturated with ethylalcohol vapor. The period of exposure was ten minutes a day and the experiment consisted of ten exposures.

The first series consisted of ten rats which had been observed to run a regular estrous cycle for several months prior to the experiment. Treatment with the alcohol vapor caused the stage of cell cornification in the vagina to persist for periods up to five days beyond normal in eight of the ten animals. One rat's cycle was unaffected and one ceased to run a cycle. In most cases there was also a tendency for the diestrous phase to be shortened. The irregularities of the normal rhythm persisted for varying lengths of time after the cessation of treatment.

The second series consisted of six rats, apparently in excellent condition, but not showing any estrous changes in their vaginal smear for periods ranging from three to six weeks. Five of these showed the typical cornified cells within three days after the initial treatment. The sixth animal was unaffected. The possibility exists that these changes were spontaneous, but their appearance so shortly after treatment is suggestive.

None of the animals lost weight during the experiment and, beyond a slight grogginess and optic irritation, were apparently unaffected.

55. *A study of the histology of the anterior pituitary of the foetal pig, with reference to growth and maturity.* W. O. Nelson (introduced by J. F. Gudernatsch), Department of Biology, New York University.

Dortzbach and Smith ('29) have studied a graded series of foetal pig anterior lobes by physiological tests and have demonstrated that an age difference exists in the appearance of the growth and maturity hormones. Anterior-lobe material from 10-cm. pigs (crown-rump measurement) evoke a response to the growth test, while the 20-cm. stage is the earliest at which sufficient maturity hormone is present to produce a positive reaction to their test.

A histological study of these stages has been made to ascertain the presence of any histological differences. The pituitaries were fixed in Zenker's fluid and stained with either haematoxylin-eosin or Nacht-Romanowsky stain. Anterior pituitaries from 10-cm. pigs were predominantly basophilic in nature, while in 20-cm. anterior lobes an eosinophilic dominance was noted. Further work in obtaining a graded series from an early age up to birth is in progress.

56. *The orientation of the early rat embryo in the uterus.* J. S. NICHOLAS, Osborn Zoölogical Laboratory, Yale University.

Following a series of experiments in which the various stages of the rat fetus have been used in testing regenerative capacity, experimental studies are being performed in the earlier embryonic stages. To determine the orientation, stages, comparable to Huber's egg cylinders of the seventh and eighth days, were used as a starting-point and then later embryonic stages added.

The embryos are definitely oriented in the uterus during the critical stages of their formation. This extends from the egg-cylinder stage to the stage of the elongation of the body stalk. During this time, the early definitive parts of the embryo are laid down and are clearly marked at the termination of the period ending about the close of the tenth day. After this period, the embryo is irregularly oriented and rotates within the amniotic sac, but at the same time it has increased in size so that its parts can be located for operation. Huber finds that the egg cylinder is oriented with its long axis across the uterus, the ectoplacental cone being directed toward the mesometrium. The dissected series shows that the egg cylinder is always located in the lateral third of the uterine swelling and that it is approximately in the median plane.

57. *The mechanism of hatching in the marbled salamander.* G. K. NOBLE AND M. K. BRADY, The American Museum of Natural History.

Amblystoma opacum deposits its eggs on land, either under leaf mold in depressions which later become temporary pools or under débris on pond banks. Eggs collected October 4th and kept at room temperature, on damp sand, from November 4th, remained viable until March 4th. Most eggs in the series kept on saturated sand hatched during this period, while no eggs in the series kept on damp sand hatched. Immersion in water is not the immediate cause of hatching, as has been hitherto assumed. Immersed eggs hatched over a period of seventy-six days, the larvae emerging from these eggs ranging from the early balancer stage to a stage in which the balancers were lost.

Hatching is dependent on unicellular glands covering the upper surface of the head. Pilocarpine hydrochloride in 1 per cent solution causes larvae beyond the rudimentary-balancer stage to hatch within two hours. Head integument containing these glands digests isolated egg capsules dipped in the pilocarpine solution, while integument from the body or tail has not this effect. Many of the glands become reduced in size during hatching.

Besides the vitelline membrane, there are two capsules separated by an absorbent jelly. Hatching in pilocarpine may be a passive digesting of the egg capsules. Usually, however, the larva is active and frequently emerges tail first, the capsules being weakened by secretions of the head glands.

58. *The induction of ovulation in salamanders by anterior-pituitary transplants.* G. K. NOBLE AND L. B. RICHARDS, The American Museum of Natural History.

As we reported previously, fresh anterior-pituitary transplants will induce egg laying in Eurycea bislineata several months before the normal breeding season, May and June. Nineteen out of twenty-four treated females laid eggs during

November, December, and January. None of the nine controls implanted with muscle laid eggs nor did any untreated salamanders. On the average it required only two transplants of anterior-pituitary substance given on alternate days to induce egg laying. Seven females receiving approximately one transplant a week for eight weeks failed to lay.

The spermathecae of Eurycea bislineata frequently contain spermatozoa in the fall. Many of the eggs laid by isolated female salamanders treated with pituitary developed normally into larvae.

It was previously found that eggs laid by salamanders receiving pituitary transplants were attached to stones in glass dishes in the manner characteristic of the species in their native brooks. This opened the possibility of studying the species characters of eggs previously unknown. Ovulation was induced in Desmognathus fuscus auriculatus during February and March. The previously undescribed eggs of this race were found to be similar to those of D. f. fuscus. In two cases nine transplants were given; in a third, six. None of the three controls receiving muscle or the many untreated females laid eggs.

59. *A metamorphic change produced in Siren by thyroxin injections.* G. K. NOBLE AND L. B. RICHARDS, The American Museum of Natural History.

The injection on alternate days of 0.2 cc. of 1: 1000 solution of thyroxin in a Siren 57 mm. in length induced metamorphic changes in this perennibranch. Eleven days after the first treatment the gills began to atrophy and the tail fin to reduce. On the twentieth day, the skin was shed in three pieces. Integument preserved two days later showed a well-developed stratum corneum and a great reduction in the number of Leydig cells. Controls injected with normal salt solution of the same amount showed no changes in gills, fins, nor integument. A Siren 65 mm. in length underwent the same changes in gills and fins following thyroxin injections. Skin shedding occurred the twenty-second day.

Injections of 0.1 cc. of the thyroxin solution in a Siren 45 mm. long induced fin and gill absorption, beginning the eighteenth day. The skin was never shed in large pieces, although integument preserved the forty-second day showed a stratum corneum and a great reduction of the Leydig cells.

Adult Sirens have a completely metamorphosed skin (Wilder), but individuals 195 mm. long retain the Leydig cells and cuticle of young larvae. Thyroxin injection produces a precocious metamorphosis of the skin of Siren, but has no effect on skull or hyobranchial apparatus. The latter remains that of a larva, in spite of the gill reduction.

60. *The ganglia of the thyroid.* JOSÉ F. NONIDEZ, Cornell University Medical College.

Contrary to current belief, the thyroid nerves show ganglionic swellings containing well-differentiated neurones. The latter were stained with the method of Cajal in puppies (newborn to five months old). As many as seven ganglia of diverse size may occur in a thyroid lobe. The larger ganglia contain from eighteen to twenty-two neurones. There are also scattered neurones among the follicles and perivascular neurones applied against the arteries. The neurones of the ganglia are rather small, have long dendrites, and slow well-developed neurofibrils. The dendrites extend into the nerves, but otherwise they do not leave the ganglia. In some neurones the axones are also stained. The neurones are enclosed within capsules. In newborn puppies there are also small, apparently undifferentiated neurones, regarded as elements in the neuroblastic phase. They are no longer present in older puppies.

Connections between myelinated fibers in the thyroid nerves and the neurones are found in most ganglia. They are represented by collaterals and terminals of the fibers wrapped around the neurone bodies (pericellular nests). Most myelinated fibers enter the gland in the superior thyroid nerve (branch of the superior laryngeal). The myelinated fibers usually form loops within the ganglia and in many cases they are recurrent. Double innervation of the thyroid (sympathetic and parasympathetic) is suggested by these findings.

61. *The effect of lesions of the superior colliculi on the light-discrimination habit in the rat.* James W. Papez and G. LaVerne Freeman, Cornell University, Ithaca.

In one series of ten rats small lesions were made in the superior colliculi. The rats were then tested in the Yerkes maze for the formation of the light-discrimination habit. The rats with more severe lesions showed a slightly greater number of trials and errors.

A second series of ten rats was first trained in the same box, and then larger lesions were made in the superior colliculi. Relearning was then carried out, with the result that some of the animals showed a marked retardation in relearning the light-discrimination maze. They showed a significant increase in both trials and errors.

At autopsy the locus and extent of the lesion in each animal were estimated. The brains were then prepared for sectioning according to the Marchi method. Those of five of the rats showing the most retardation following the lesion have been sectioned. They show a double degeneration of the tectospinal tracts, severe injury to the nuclei in the region of the posterior commissure, including the habenulopeduncular tracts. The indications are that lesions of these parts tend to produce a definite retardation of the light-discrimination habits in the rat.

62. *Thioneine and amide nitrogen of the blood of adrenalectomized and nephrectomized cats.* W. M. Parkins, W. W. Swingle, and J. J. Pfiffner, Biological Laboratory, Princeton University.

Normal cats under constant laboratory conditions of diet, temperature, exercise, etc., show over a period of thirty-one days a consistent gradual decrease of thioneine content of the blood. The average figures per 100 cc. were 4.08 mg. on the first day and 2.35 mg. on the thirty-first day. The thioneine content of the blood of adrenalectomized cats (six) showing marked symptoms is comparatively normal. The fluctuations are within the range of normal physiological variation. The thioneine content of the blood of nephrectomized cats (four) is consistently low, showing a decrease in two to five and one-half days comparable to the decrease found in normal unoperated cats over a period of thirty-one days. In view of the possible physiological variations and the comparatively low content of the blood of normal cats, perhaps little if any of the decrease found in nephrectomized cats could be attributed to the absence of kidney function.

The amide-nitrogen content of the blood of normal cats (twenty) ranged between 143 mg. and 120 mg., the average being 132 mg. per 100 cc. of blood. The blood amide nitrogen of adrenalectomized cats remains remarkably constant, showing a slight tendency to increase in a few cases. Amide nitrogen of the blood of nephrectomized cats decreases, the average drop being from 132 to 116 mg. per 100 cc. of blood.

63. *Secondary vestibular tracts in the cat.* A. T. RASMUSSEN, Department of Anatomy, University of Minnesota.

In the light of some recent reports on the vestibular tracts, which disagree in certain respects with older studies by others and with unpublished experiments performed some years ago in our laboratory, a restudy of the degeneration resulting from small mechanical lesions in the vestibular nuclei of cats has been made. The results so far agree with those of others in the following respects: 1) The sujerior vestibular nucleus sends fibers up the medial longitudinal fasciculus (m. l. f.) of the same side; 2) the lateral vestibular nucleus, down the ventral funiculus of the spinal cord of same side; 3) the medial (triangular) vestibular nucleus, both up and down in the opposite m. l. f.; 4) the descending vestibular nucleus (essentially a continuation of the medial), down in the opposite m. l. f.; and, 5) also down the m. l. f. of the same side, which has been denied. On this latter point we depend for evidence on small dorsal lesion strictly within the vestibular nucleus just below the stria medullaris acustica, with no involvement of the reticular formation. 6) The fibers from nucleus fastigii are largely crossed and some seem to continue with the fibers from the lateral vestibular nucleus farther down the spinal cord than is generally admitted. 7) Since most of our lesions were through the cerebellum, there is further evidence that the cortex of the vermis sends fibers mostly to the opposite nucleus fastigii.

64. *Studies on the mesencephalic nucleus of the trigeminal nerve. a. The relation of the mesencephalic nucleus to the eye-muscle nerves in the dog. b. Types of cells in the mesencephalic nucleus of the dog based on Nissl-granule arrangement.* JOHN J. SHEININ (introduced by Sam L. Clark), Northwestern University Medical School.

a. The extra-ocular muscles were carefully identified in unilateral extirpation of the orbital contents of three adult dogs. The animals were sacrified two weeks after the operation and the mesencephalic nuclei were studied in toluidin-blue preparations. Only occasionally were chromatolyzed large globoid cells observed. It is possible that these few may be considered as a normal occurrence, since occasional ones are found in normal sensory ganglia (Windle, '27). These results suggest that the large globoid cells of the mesencephalic nucleus send few if any fibers to the extra-ocular muscles.

b. The cells of the mesencephalic nucleus and locus caeruleus in the dog were studied in toluidin-blue preparations. Based on Nissl-granule arrangement, four definite types of cells were identified in the mesencephalic nucleus. Three of these types, which constitute about 50 per cent of the total number of cells in the mesencephalic nucleus, have not hitherto been described in this nucleus. All types of cells identified in the mesencephalic nucleus resemble those described in the semilunar and dorsal root ganglia. Pigmentation of cells was not limited to the extent of the locus caeruleus, but was observed in the nuclei of third and fourth cranial nerves; the red nucleus; the motor, main sensory, and mesencephalic nucleus of the trigeminal nerve.

65. *Cyclical changes in the vaginal epithelium, studied by the method of biopsy.* Bertram G. Smith and Endre Brunner, Department of Anatomy and Gynaecological Clinic, University and Bellevue Hospital Medical College, New York University.

Material is being obtained from patients with known menstrual histories for the purpose of determining more accurately the extent and history of the peculiar cyclical differentiation of the vaginal epithelium known to occur in man as well as in some of the lower mammals. The results thus far indicate a widespread distribution and regular occurrence of the cyclical changes in patients with normal menstrual histories. Sections illustrating some phases of the cycle will be demonstrated.

66. *Normal variations in red-blood-cell counts over long periods of time.* Christianna Smith, Department of Zoölogy, Mount Holyoke College.

A series of total red-blood-cell counts over long periods of time is being made to determine the normal variations of individual women from day to day. Four women have been studied to date; three series have been completed (fifty-two days, fifty-eight days, seventy-nine days); one series is in progress (forty days). The more accurate and steady the technique, the more apparent the pattern of the curve. Two series show fairly regular depressions appearing at about ten- to fifteen-day intervals; the other two series suggest the same pattern, but parts are more irregular. The menstrual series seem to be a part of the general pattern. This might explain the different results given for menstrual periods in the literature. Two menstrual periods included in the fifty-two-day series followed the same trend, a decreasing one during the period, found for six previous series of seven- to nine-day counts of isolated menstrual periods of the same individual. Other individuals showed an increasing count during the period.

67. *The influence of thyroidectomy upon the amount of gonadal-stimulating hormone present in the anterior hypophysis.* Philip E. Smith and Earl T. Engle, Department of Anatomy, College of Physicians and Surgeons, Columbia University.

Castration, as first shown by Engle and later confirmed by Evans and Simpson, markedly increases the content of the gonadal-stimulating hormone of the anterior hypophysis. This finding makes it of interest to determine if the ablation of an endocrine gland having a close interrelation to the hypophysis but not to the gonads would also influence the amount of hormone stimulating gonadal development in the anterior hypophysis. We therefore made implants, into immature mice, of anterior hypophyses from female rats which had been thyroidectomized in excess of 200 days and from their normal litter-mate controls. No constant difference in the response of the gonads or uterus was induced by implants from the two types of donors. In certain cases the implants from the thyroidless animals were somewhat more effective, in others they were less effective than those from the normal donors. That thyroidectomy would not upset the pituitary content of the gonadal-stimulating hormone would be anticipated from the fact that thyroid ablation in rats does not cause a severe upset of the reproductive system.

68. *Parallelism in the interosseous muscles of primate hand and foot.* WILLIAM L. STRAUS, JR., Department of Anatomy, Johns Hopkins University.

The interosseous muscles of the primate hand exhibit a tendency to parallel those of the foot in their orientation. The primitive condition in both extremities is a grouping with reference to the medius, the longest digit. This arrangement occurs in all New and Old World monkeys. In gibbon, orang, and chimpanzee the axis of orientation is always through third finger and usually through third toe, occasionally through second toe. The gorilla always has the axis through third finger and usually through second toe, occasionally through third toe. In man the grouping is around third finger and second toe; rarely, however, the axis passes through the index finger, the arrangement in the hand thus paralleling that in the foot. This is possibly correlated with the human lengthening of the second finger (relative to third), just as the second toe is lengthened (relative to third). The prosimians frequently exhibit a shift of the interosseal axis to fourth finger and fourth toe, both of which are usually the longest digits of their respective extremities. There is therefore a rough correlation between interosseal axis and digit length.

Both hand and foot of a particular genus may exhibit parallel specializations in axial shift, but the foot tends to be more advanced than the hand. This is shown by conditions in certain genera of prosimians and in anthropoids and man.

69. *Persistence of subcentral vein in adult man; developmental permutations of the components of lumbar vena azygos.* LEON H. STRONG, Anatomical Laboratory, University of Michigan Medical School.

In a study of fifty-four cadavers the lumbar vena azygos proved to be of two main types and their combinations. In one type the vessel was situated dorsal to the lumbar arteries, thus indicating a derivation from the dorsal aortic plexus (Strong, '27, '28). The chief longitudinal derivative of this plexus has been designated in man, subcentral vein (Reagan, '27). In the other type, the vessel was situated ventral to the lumbar arteries and thus represents the lumbar part of the medial sympathetic vein of Reagan (supracardinal of Huntington and McClure).

Remnants of the subcentral vein occurred on one or both sides in forty-one cadavers. Five of these were so large as to be of azygos caliber, and thus were of surgical importance. Functional derivatives of the dorsal aortic plexus drain the aorta, vena cava inferior, cisterna chyli, lymph glands, some prevertebral ganglia, the sympathetic trunk, and crura of the diaphragm.

The lumbar part of the medial sympathetic vein was represented in thirty-five cases. On the right side it regularly anastomosed with the vena cava inferior. On the left, the vein joined the vena cava inferior in twelve cadavers, while in seventeen it formed a connection with the left renal vein. The left renal vein formed an important anastomosis with the left ascending lumbar vein in twenty-one instances.

70. Observations on the structure of the eye of the albino rat. R. M. STRONG AND HERMAN F. DE FEO, Department of Anatomy, Loyola University School of Medicine.

A careful study of the gross and histological structure of the albino-rat eye has been made. There is a relatively very large lens, but there is less differentiation in the choroid coat, sclera, ciliary body, and iris than appears in the eye of large mammals.

71. Origin of columella auris of Anura. H. N. VIOLETTE (introduced by A. W. Meyer), Department of Anatomy, Stanford University.

The columella auris of adult Rana palustris lies in relation to the hyomandibular nerve, to which the facial and glossopharyngeal nerves contribute. It has hence been considered as a derivative of the hyoid arch. In larvae of Rana palustris the columella develops at the level of the glossopharyngeal ganglion. This relationship is evident in larvae whose otic vesicles have been excised in the tail-bud stage. A pars interna forms within an inner layer of the fenestra vestibuli, containing the operculum. A pars media forms and protrudes laterally from an outer layer of the fenestral membrane which has a cellular connection with the developing ligament of the m. stapedius, on the outer surface of the operculum. It is believed that the anuran columella (plectrum) forms from an anlage caudal to the hyoid arch. This hypothesis is based on the following facts: 1) the chondroblastema undergoes migration in a cephalic direction before chondrification commences; 2) the first chondrification appears at the level of the glossopharyngeal ganglion; and, 3) the larval columella lies far posteriorly to the position of the anlage of the tube. Evidence of the relationship of the nerves to the developing columella favors its origin from the first branchial arch. This and the classic hyomandibular hypothesis are being tested by experiments.

72. Report of a case of complete absence of the pericardium in a human subject. JAMES CRAWFORD WATT, University of Toronto.

The case reported is that of a man aged fifty-two, well nourished, active, and hard-working, who died suddenly of coronary thrombosis and myocardial degeneration.

Postmortem examination disclosed a heart of normal size, but of rather longer, narrower outline than normal, lying more transversely than usual in the thoracic cavity. There was no sign whatever of a separate pericardial cavity. The heart was lying in the same cavity as the lung, with the latter wrapped well over both its anterior and posterior surfaces, only the base and the tip of the apex being uncovered by the lung.

The right phrenic nerve was in its usual position, but the left phrenic nerve lay between the chest wall and the parietal pleura a short distance lateral to the sternum. The left lung showed four lobes—superior, middle, inferior, and subcardiac.

The parietal serous membrane was reflected on to the heart at the great vessels. The transverse sinus of the pericardium was present. Reflection of the serous membrane on to the left lung occurred at the root and along a line below this off the back of the heart, which obliterated a large part of the oblique sinus of the pericardium. No folds suggestive of formation of any part of a separate pericardial wall were present.

73. A rapid method for the staining of myelin sheaths. ARTHUR WEIL (introduced by S. W. Ranson), Northwestern University Medical School.

Formalin-fixed material embedded in celloidin or paraffin. Sections cut 20 to 30 μ from celloidin and 10 to 15 μ from paraffin material. Remove paraffin. Bring sections for five minutes in 5 per cent solution of potassium bichromate. Wash twice in tap-water. Stain fifteen minutes at 50°C. in fresh mixture of equal parts of: 4 per cent solution of iron alum and 1 per cent water solution of haematoxylin (prepared from old, 10 per cent absolute alcoholic solution). Wash in tap-water. Keep in 4 per cent solution of iron alum until gray matter may be just recognized. Wash three times in tap-water. Differentiate in solution of: borax, 10 grams; potassium ferricyanide, 12.5 grams; distilled water, 1000 cc. Wash four times in tap-water; add 28 per cent ammonium hydroxide to the third wash (ten drops to 50 cc.). Alcohol, xylol, balsam. (Further details in Archives of Neurology and Psychiatry, vol. 20, p. 392, 1928.)

74. The utricular fold (or utriculo-endolymphatic valve) in the ear of a three-year-old child. JOHN GORDON WILSON AND BARRY J. ANSON, Departments of Otolaryngology and Anatomy, Northwestern University Medical School.

The fold of the utricular wall which forms the posterior boundary of the elliptical opening of the utricle into the endolymphatic duct has recently been observed in serial sections of the ear of a three-year-old child. Subsequent to Bast's original description of this structure as seen in a human embryo, the present authors discussed its occurrence in a child two years of age and in adult man.

The fold in the three-year-old exhibits only one feature in which it differs substantially from those described in our earlier accounts: the free end of the fold is but very slightly upturned at its tip, and possesses no marked enlargement.

Photomicrographs of the utricular fold in various series will be exhibited (see Demonstrations).

75. The sensory component of the spinal accessory nerve. WILLIAM F. WINDLE, Northwestern University Medical School.

The presence of groups of nerve cells of sensory type on the roots of the spinal accessory nerve in man was shown by Weigner ('01), and Fahmy ('27) described an inconstant extracranial ganglion on this nerve. The present observations show that there are groups of cells placed irregularly along the course of the accessory nerve in the cat and the monkey that conform in type to those of typical spinal ganglia. In the cat almost all of them seem to undergo chromatolysis after section of the nerve just proximal to the sternomastoid muscle. Similar changes were found in cells of the first cervical spinal ganglion and to some extent in the second, third, and fourth, but they may be partly due to the operative procedures. Degeneration in sensory cells of the accessory nerve was more marked and it is suggested that these together with some of the cells of the upper cervical ganglia mediate sensation from the sternomastoid and trapezius muscles.

76. *The effect of castration on the development of vitamin-A deficiency in the albino rat.* J. M. WOLFE AND K. E. MASON, Department of Anatomy, Vanderbilt University School of Medicine.

Previous studies (Mason, '30) showed that vitamin-A deficiency in otherwise adequate diets, supplemented with excessive amounts of vitamin E, induces testicular degeneration similar to that described (Mason, '25) as resulting from vitamin-E deficiency. These studies indicate that vitamin A, as well as the antisterility factor, is necessary for and probably utilized directly in the metabolic activities of the testis. To test this suggestion, comparisons were made between the onset of xerophthalmia in rats castrated at twenty-one to thirty days and in unoperated litter-mate controls, placed on the A-deficient diet at the same time. In a series of forty-four males, (eighteen total castrates; eight unilateral castrates; eighteen unoperated controls) both the controls and unilateral castrates of various litter groups exhibited early signs of xerophthalmia at a distinctly earlier period (variation, two to fourteen days; average, eight days) than their castrated brothers. The average time required to induce xerophthalmia in the controls on the diet used varied from eighteen to thirty-nine days in various litters, but litter-mate rats showed close uniformity. Similar results were obtained in a series of ten castrated females and ten unoperated controls. However, in both sexes the period elapsing between the early appearance of the eye symptoms and their maximum manifestation was much shorter in total castrates than in unoperated controls and unilateral castrates.

77. *The polynuclear count in the rat, with special reference to vaginal smears.* J. FRANKLIN YEAGER AND H. O. HATERIUS (introduced by Robert Chambers), Department of Biology, New York University.

Polynuclear counts have been made from blood and from vaginal smears taken simultaneously, the smears being prepared in the usual manner, Wright's blood stain being used in both instances for the most part. (In some cases Fadicit I was used for blood smears and Delafield's haematoxylin for vaginal smears.) The count included only cells possessing a distinct cytoplasmic outline.

The blood count was found to be decidedly left-handed as compared with that of normal man. The vaginal-smear counts, while also decidedly left-handed as compared with the blood count of man, were, however, somewhat right-handed when compared with the animal's own blood count. The average weighted mean of a group of thirty-five blood polynuclear counts was 1.09, with a standard error of ± 0.0016, while the average weighted mean of a group of twenty vaginal polynuclear counts was 1.69, with a standard error of ± 0.039.

The polynuclear count of the rat is similar to that reported in the cow, the sheep, and the horse (Simpson, '29).

78. *Experiments on the development of the auditory vesicle in Amblystoma.*

C. L. Yntema (introduced by R. G. Harrison), Osborn Zoölogical Laboratory, Yale University.

The ear placode of A. punctatum was grafted into the fore-limb region, and between the eye and ear, at stages from the approximation of neural folds up to the early tail bud. Degree of development of resulting ear was found to increase with advance in age of donor.

When the ear rudiment was grafted into several positions during the early head-process stage, most normal differentiation occurred when placed between the eye and ear. Elsewhere, only vesicular ears, usually with sensory areas, resulted. In the posterior trunk region an incomplete cartilaginous capsule, connected with a vertebra, occasionally developed.

Ectoderm from posterior to the ear rudiment, when placed in the ear region during the late neural-plate stage, sometimes formed functional labyrinths. Shortly after this stage, power to form an ear from this ectoderm was lost. Transplantation of foreign ectoderm into the ear region when the neural folds were approximating showed that its potency to form an ear varied with its distance in a given direction from the ear region.

79. *A study of the motility and resistance of rat spermatozoa at different levels in the reproductive tract.*

Donald E. Yochem (introduced by L. B. Arey), Northwestern University Medical School.

When both testes are removed, and the spermatozoa in the head and tail of the epididymis and ductus deferens are isolated with ligatures and confined within the abdomen, the maximum period of survival is as follows: tail of the epididymis, eight days; ductus deferens, seven days; and head of the epididymis, six days. Isolation as described, but retention in the scrotum, almost doubles the above time. The deleterious effects of abdominal temperature on spermatozoan life is again demonstrated. This confirms the results of Moore ('26), Heller ('28), and others. Temperature effects on sperm in vitro, equivalent to abdominal (34°C.) and scrotal (31°C.) temperature, are similar to those in vivo, but the survival time is only a few hours.

Spermatozoa removed from the reproductive tract and placed in saline solution show that the intensity of motility increases from the testis to the tail of the epididymis, where a maximum is attained; in the ductus deferens there is no recognizable further increase in their activity. Likewise, there is a progressive increase in the relative number of motile spermatozoa toward the tail of the epididymis. The resistance of the spermatozoa to alkalinity or acidity increases from the testis to the tail of the epididymis, then decreases in the ductus deferens. At 2°C. there is a decrease in resistance from the testis to the tail of the epididymis and ductus deferens.

80. *Destruction and new formation of thyroid follicles in the dog.* **Gustav Zechel** (introduced by Otto F. Kampmeier), Department of Anatomy, College of Medicine, University of Illinois.

The normal thyroid gland of the dog consists of follicles varying in size and number of cells. Some follicles are composed of few cells with a very small cavity, and there are others in the form of cell groups arranged in roundish masses without any lumen. The latter are always congregated and create the impression of a locus of newly developing follicles. The colloid of the normal gland has a strong affinity for eosin and stains a bright red. The colloid of some follicles does not stain with eosin as readily as that of others, such even staining with haematoxylin. The follicles of the latter character usually show a shape which is not as spherical as the type containing the bright red colloid, and tend to assume a more irregular outline. In certain spots of the thyroid gland the follicles are flattened, almost free of colloid, and are separated by proliferating connective tissue—all of this suggesting a picture of degeneration and the resorption and elimination of the old non-functioning follicles. This hypothesis is supported by experimental partial resection of the gland. Some time after operation, during which the anterior half of the thyroid gland was removed on each side, the areas of small follicles and cell masses lacking cavities grow more numerous and larger, so that these become prevailing areas in section and therefore might be considered as the regenerating centers of the thyroid gland. Due to the fact that there appear to be centers of regeneration in a normal thyroid of the dog on one hand and centers of degeneration on the other, we may believe that there is a constant formation of new follicles and a destruction of old ones. These data obtained in preliminary studies would lead to the conclusion that there is a follicular life cycle of the thyroid gland of the dog.

DEMONSTRATIONS

1. *Anomalous mouse embryos at end of first day in life of embryo proper (eight days, gestation age).* **Ezra Allen**, New York Homeopathic Medical College and Flower Hospital.
2. *Sections of tissues from a 25-cm. (C. R.) human fetus stained by various methods.* **Ezra Allen, Hadley Kirkman, and Irene Kirkman**, New York Homeopathic Medical College and Flower Hospital.
3. *Motion-picture records showing the typical stages in the development of muscular activity in albino-rat fetuses which are used in connection with the corresponding changes in the nervous system.* **A. W. Angulo y González**, The Wistar Institute of Anatomy and Biology.
4. *The endolymphatic duct system in the ear of a two-year-old child.* **Barry J. Anson and John Gordon Wilson**, Northwestern University Medical School.
5. *Anatomical and physiological study of the mesial, dorsal, and lateral surfaces of the cortex of the sheep brain caudal to the cruciate sulcus; the normal histological structure of the cortex and Marchi and Weigert preparations after extirpation of the various gyri.* **Charles Bagley, Jr.**, Neurological Laboratory of the Phipps Psychiatric Clinic, Johns Hopkins University.
6. *An early menstrual repair stage.* **G. W. Bartelmez**, Department of Anatomy, University of Chicago.

7. *a) Contribution to the knowledge of the direction of flow of blood in the cardiac vessels. b) A technic for producing metal 'corrosions.'* O. V. BATSON, Graduate School of Medicine, University of Pennsylvania.
8. *Golgi bodies, vacuome, and mitochondria in the salivary glands of the Chironomus larva.* H. W. BEAMS AND J. B. GOLDSMITH (introduced by H. E. Jordan), Laboratory of Histology and Embryology, Medical School, University of Virginia, and the Department of Zoölogy, University of Wisconsin.
9. *Charts of the stature, sitting height, sitting-height index, head length, head breadth, cephalic index, eye color, and hair color of about 3000 Old Virginians of both sexes from six to sixty years of age. Averages and probable errors. Also comparisons with many peoples throughout the world.* ROBERT BENNETT BEAN, School of Anatomy, University of Virginia.
10. *Charts and curves illustrating neonatal decrease and recovery of thymus weight in man.* EDITH BOYD, Institute of Child Welfare and Department of Anatomy, University of Minnesota.
11. *A quantitative analytic comparison of the prediction value of various formulae for surface area, including two formulae derived by the method of least squares and several expressions based upon height and weight derived by the geometric method of Du Bois.* EDITH BOYD, Institute of Child Welfare and Department of Anatomy, University of Minnesota.
12. *Effect of placental extracts on ovarian-stimulating properties of anterior hypophysis.* JOHN C. BURCH AND R. S. CUNNINGHAM, Vanderbilt University School of Medicine.
13. *The process of transformation from male to female in the gonads of parabiotic Amblystoma.* ROBERT K. BURNS, JR., Department of Anatomy, University of Rochester.
14. *Roentgenograms of developing experimental scoliosis of both the functional and structural types in white rats and guinea-pigs.* E. J. CAREY, Marquette University School of Medicine.
15. *Further developments of the method for studying living cells and tissues in transparent chambers inserted in the ears of rabbits.* E. R. CLARK, H. T. KIRBY-SMITH, R. O. REX, AND R. G. WILLIAMS, Department of Anatomy, University of Pennsylvania.
16. *Innervation of the pia mater of the spinal cord.* SAM L. CLARK, Northwestern University Medical School.
17. *Effects of ultraviolet radiation upon the integument of the urodele, Triturus viridescens.* H. H. COLLINS AND E. A. WOLF, University of Pittsburgh.
18. *The use of a modification of the Spalteholtz method in the study of regeneration in the urodele, Triturus viridescens.* S. H. COOKE (introduced by H. H. Collins), University of Pittsburgh.
19. *Specimen illustrating growth and lactation of the mammary gland in spayed virgin rabbits, produced by alkaline extracts of the anterior lobe of the hypophysis.* GEORGE W. CORNER, Department of Anatomy, University of Rochester.
20. *Paraffin and celloidin sections of formalin-hardened tissue stained with silver nitrate.* H. A. DAVENPORT (introduced by S. W. Ranson), Northwestern University Medical School.
21. *1930 bookrack for McClintic dissecting tables.* J. M. ESSENBERG (introduced by R. M. Strong), Loyola University School of Medicine.

22. *The presence and absence of elastic tissue in the arteries of different vertebrates as shown by elastic-tissue stains.* S. H. Gage, Cornell University, Ithaca.
23. *Physiological reactions of goldfish with severed spinal cord.* Davenport Hooker, Department of Anatomy, University of Pittsburgh.
24. *Congenital hydrocephalus in calf and rat. Skull and brain.* J. M. Houck (introduced by H. E. Jordan), Laboratory of Histology and Embryology, Medical School, University of Virginia.
25. *I. Transformation of the modified ('freemartin') ovary of Amblystoma tigrinum into a functional testis. Dissections and microscopic preparations.* R. R. Humphrey, University of Buffalo.
26. *A modification of the Schultz method used in the study of the skeletal system of the urodele, Triturus viridescens.* Marie Ignelzi (introduced by H. H. Collins), University of Pittsburgh.
27. *Slides and photomicrographs illustrating functional changes in sympathetic-ganglion cells in the albino rat.* E. H. Ingersoll (introduced by A. Kuntz), Medical College of Virginia.
28. *The anatomy of the female pelvis: a cinematographic presentation with sound.* Howard B. Kellogg and William F. Windle, Northwestern University Medical School.
29. *Sections of ovaries of hermaphroditic and pseudohermaphroditic children.* S. I. Kornhauser, School of Medicine, University of Louisville.
30. *Moving pictures of normal and malignant cells.* Warren H. Lewis, Department of Embryology, Carnegie Institution of Washington, Baltimore.
31. *External and internal anatomy of the forebrain of the opossum.* Y. T. Loo (introduced by C. J. Herrick), University of Chicago.
32. *Biological researches with the ultraviolet microscope illustrating recent developments by optical sectioning.* Francis F. Lucas and Mary B. Stark, Bell Telephone Laboratories and Flower Hospital.
33. *X-ray films of lipiodolized human bronchi and bronchioli, affording a comparison of the stages of full expiration and full inspiration.* C. C. Macklin and P. M. Andrus, Department of Anatomy, University of Western Ontario, and The Queen Alexandra Sanitorium.
34. *Microscopic sections and photomicrographs of liver tissue from animals whose portal blood contained unusual amounts of water.* Madge T. Macklin and C. C. Macklin, Department of Anatomy, University of Western Ontario.
35. *The dilution of portal blood with water.* Madge T. Macklin and C. C. Macklin, University of Western Ontario.
36. *Dissected specimens, photographs, and drawings of a hitherto undescribed insertion of the temporal muscle.* G. J. Noback, Department of Anatomy, New York University.
37. *The mechanism of hatching in the marbled salamander.* G. K. Noble and M. K. Brady, The American Museum of Natural History.
38. *A metamorphic change produced in Siren by thyroxin injections.* G. K. Noble and L. B. Richards, The American Museum of Natural History.
39. *Motion pictures illustrating various postural and locomotor reactions.* S. W. Ranson and J. C. Hinsey, Northwestern University Medical School.
40. *Material illustrating the prenatal growth of the musculature, skin, and adipose tissue of the human body.* Harold E. Roe (introduced by R. E. Scammon), Department of Anatomy, University of Minnesota.

41. *Charts, curves, and graphs illustrating the growth and involution of the great or frontal fontanelle.* R. E. SCAMMON, Institute of Child Welfare and Department of Anatomy, University of Minnesota.
42. *Diagrams, graphs, and tables illustrating the variation in specific gravity of the brain of the albino rat from birth to three years of age.* C. G. SMITH (introduced by C. C. Macklin), Department of Anatomy, University of Western Ontario.
43. *Sections of the epithelium of the human vagina, illustrating some phases of the cyclical history.* BERTRAM G. SMITH AND ENDRE BRUNNER, University and Bellevue Hospital Medical College, New York University.
44. *Use of the x-ray in embryology.* WALTER E. SULLIVAN, Anatomical Laboratory, University of Wisconsin.
45. *Motion pictures of living mammalian embryos and fetuses.* ENGELBREKT A. SWENSON, Department of Anatomy, University of Pennsylvania Medical School.
46. *Graphs, curves, and tables illustrating the changes in head form in the first ten days after birth.* LEON J. TIBER (introduced by R. E. Scammon), Department of Anatomy and Department of Obstetrics and Gynecology, University of Minnesota.
47. *A rapid method for staining myelin sheaths.* A. WEIL (introduced by S. W. Ranson), Northwestern University Medical School.
48. *The architecture of the tunica media of arteries.* J. D. WEINTRAUB (introduced by O. V. Batson), Graduate School of Medicine, University of Pennsylvania.
49. *Bielschowsky preparations illustrating the innervation of the eye muscles.* H. J. WILKINSON (introduced by S. W. Ranson), Northwestern University Medical School.
50. *Nerve endings in the human skin, with charts showing the distribution and relative depths of the various types of endings in a selected area.* ROY G. WILLIAMS (introduced by E. R. Clark), Department of Anatomy, University of Pennsylvania.
51. *The utricular fold (or utriculo-endolymphatic valve) in the ear of a three-year-old child.* JOHN GORDON WILSON AND BARRY J. ANSON, Northwestern University Medical School.

PAPERS TO BE PRESENTED

at the

JOINT SESSION

of the

AMERICAN ASSOCIATION OF ANATOMISTS

and

AMERICAN ASSOCIATION OF PHYSICAL ANTHROPOLOGISTS

1. *Old Virginians.* ROBERT BENNETT BEAN, School of Anatomy, University of Virginia.

Examinations and measurements have been made during the past twelve years of more than 3000 male and female children and adults between the ages of six and sixty years, who for three generations have been residents of Virginia. This was done to establish a standard, for future comparison, of stocks that had been in one environment for a considerable time. Statistical tables and charts are being prepared to show the stature, sitting height, sitting-height index, head length, head breadth, cephalic index, hair color, and eye color of each sex at each age from six to sixty years. Already the stature shows that Old Virginians are the tallest people in the world, taken as a whole group, and about of the same stature as Hrdlička's Old Americans. A few Patagonian giants, a few East African negroes, and a few Scotch farmers are a little taller, but no whole group of any of these peoples is taller, or as tall. A group of Old Virginians from Albemarle County, farmers of the landlord type, averaged over 180.0 cm., or 6 feet. They represent the pioneer gentry, but they cannot be taken as characteristic of the group of Old Virginians, any more than the Patagonian giants, East African negroes, or Scotch farmers can be taken as characteristic of the whole people in these countries. Other facts will be given at the meeting.

2. *A comparison of the limbs, hands, and feet of man, anthropoid apes, and primitive Eocene mammals.* WILLIAM K. GREGORY, The American Museum of Natural History.

Reviews points of agreement between Prof. H. F. Osborn's version and the Keith-Gregory version of man's ascent from lower primates. Takes up Osborn's suggestion that hands and feet of man may be derived, independently of those of specialized apes, from Eocene mammals with divergent pollex and hallux.

Shows that in many characters of the humerus, manus, pes, etc., man is far nearer to modern gorilla and chimpanzee than to known Eocene mammals. Shows, *a*) that the mountain gorilla, although now spending much time on the ground, has not yet wholly passed from the brachiating stage; *b*) that it nevertheless has especially human characters in all its girdle and limb bones. Infers that human-like characters are not inconsistent with remote origin from brachiating ancestors.

3. Form types of the body and in osteology. ALEŠ HRDLIČKA, United States National Museum.

Besides the ordinary range of normal variation in the human body and its different parts, there is observable another phenomenon of importance which may be called typogeny. This process may be defined as the tendency of the variations of grouping themselves into more or less definite form types.

Such variations are very perceptible in the body as a whole, a subject reported upon repeatedly by Bean; and they are very clearly defined in many parts of the human skeleton.

Many of these variations are connected with muscular peculiarities, and are influenced by muscular action. In the majority of cases they may be regarded therefore as essentially functional. But some of them are hereditary and have evidently a deeper meaning.

4. Rôle of structural variations in static foot disorder. DUDLEY J. MORTON, Department of Anatomy, College of Physicians and Surgeons.

Certain anatomical variations of the feet are specifically causal to static disorders under conditions imposed by our civilized environment; namely: 1) Short first metatarsal bone as determined by a rearward position of its head in relation to that of the second metatarsal bone. 2) Sesamoid bones located posteriorly toward the neck of the first metatarsal, instead of being centered beneath the head of that bone. 3) Hypermobility of the hallucial segment (first metatarsal and its cuneiform bone) as the result of ligamentous laxity between the navicular, the inner cuneiform, and middle cuneiform bones.

These conditions may exist singly or in combination. They vary in degree in different feet from zero to a conspicuous morphological character as revealed by dorsoplantar x-ray examination. Each of them imposes an increase in the burden of body weight upon the second metatarsal bone, according to the greater degree of such variations.

The increased burden upon the second metatarsal becomes expressed in a fourth anatomical character—a distinct, and sometimes conspicuous, hypertrophic development of that bone; the amount of this enlargement is determined by, 1) the totality of causal variations, 2) age of the individual, and, 3) habitual functional demands. Most conspicuous instances of this hypertrophy are found either in middle-aged persons, or in younger adults presenting a marked degree of the causal variations listed above.

Such structural variations are of themselves merely potential causes of foot disorder, since they undoubtedly prevail as widely among rural groups and non-shoe-wearing races as among our urban populations, but are not accompanied by disabling symptoms. Hard, level pavements and floors, poorly designed shoes, excessive occupational demands upon feet, and other artificial conditions induced by our civilized mode of life all combine to furnish the supplementary environmental factor whereby these variations—as structural imperfections—are changed from a potential to an actual source of foot disability.

5. *Human variability compared with the variability of other primates.* ADOLPH H. SCHULTZ, Laboratory of Physical Anthropology, Johns Hopkins University.

The number of vertebrae in the various spinal regions is much more variable in anthropoids than in man. For instance, thoracolumbar vertebrae deviate from the statistical norm in only 8 per cent of 3856 human spines, but in 42 per cent of eighty-six gorilla spines. In all higher primates the number of sacral vertebrae varies more than the number of thoracic or of lumbar vertebrae and the coccygeal vertebrae are most variable. The praecaudal spine of man shows a normal combination of variations (12 thoracic, 5 lumbar, 5 sacral) in 71 per cent of the cases, that of orang (12, 4, 5) in only 46 per cent, and that of chimpanzee (13, 4, 5) in only 35 per cent.

According to their variation coefficients, the proportions between the lengths of the limb bones show approximately equal stability in man and other higher primates.

Variations in the two halves of the body (asymmetries) are more pronounced in man than in anthropoids. The limb bones of lower primates are as frequently longer on the right side as they are longer on the left. Among higher primates the upper extremity is most frequently longer on the right than on the left in gibbon, orang, and, particularly, man, whereas the lower extremity is most frequently longer on the left side in chimpanzee and in man. The clavicles show greater asymmetries than the limb bones.

6. *The contributions to our knowledge of the growth and development of the fetus, infant, and child, 1920–1930: A quantitative summary.* RICHARD E. SCAMMON, Institute of Child Welfare and Department of Anatomy, University of Minnesota.

A quantitative analysis has been made of some 1800 publications on the growth and development of the body in the fetal period, infancy, childhood, and adolescence that have appeared in the last ten years. These have been classified according to subject matter, method of study, language of publication, and vehicle of publication. An analysis has also been made of the sources of literature on these subjects that have developed in the last decade, the chief bibliographic sources that have appeared in these years, and the development of analytic and quantitative methods in this period. A comparison is made with results of a similar analysis of studies appearing in the decade 1900–1910 and of those of fifty years ago, 1870–1880.

7. *The adolescent lag in the human skeleton.* T. WINGATE TODD, Western Reserve University.

1. The dates of epiphysial union and eruption of permanent teeth are related so that they may be regarded, for this purpose, as different aspects of the same

phenomenon. Both order-pattern and date of union or eruption can be and are modified by pathological conditions of the body.

2. There is a chronological linkage for both epiphysial union and eruption of teeth which is normally stable for a particular animal, but varies with the order or genus.

3. The special character of the primates is a lag of maturation throughout infancy and childhood. Nevertheless, even in them adult characters quickly reach complete development once they have appeared.

4. The particular feature of man's development is his adolescent lag, and the scatter in individual variation which is most pronounced between eleven and fifteen years. The most stable and uniform periods of human skeletal development for both males and females are between one and five years and between eighteen and twenty-two years.

PROCEEDINGS OF THE AMERICAN ASSOCIATION OF ANATOMISTS

FORTY-SIXTH SESSION

University of Virginia School of Medicine, Charlottesville, Virginia, April 17, 18, and 19, 1930

THURSDAY, APRIL 17, 1930

10.00 a.m. The Forty-sixth Session of the American Association of Anatomists was called to order by President Charles R. Stockard, who appointed the following committees:

Auditing Committee: Dr. Carl C. Speidel and Dr. Leslie B. Arey.

Nominating Committee: Dr. Harvey E. Jordan, Chairman; Dr. Walter E. Sullivan, and Dr. Charles V. Morrill.

The remainder of the session was devoted to the presentation of scientific papers.

FRIDAY, APRIL 18, 1930

12 noon. Association Business Meeting, with President Charles R. Stockard presiding. The Secretary reported that the minutes for the forty-fifth session were printed in full in The Anatomical Record, volume 42, no. 2, pp. 189–194, inclusive. On motion, seconded and carried, the minutes of the forty-fifth session were approved by the Association as printed in The Anatomical Record.

THE ANATOMICAL RECORD, VOL. 45, NO. 4
MAY, 1930

Report of the Treasurer: The Treasurer made the following report for the year 1929:

Balance on hand, December 31, 1928 (last audit of accounts)		$1,080.95	
Receipts from dues and abstracts, 1929		520.83	
Interest on deposits:			
6/28/29	$11.88		
12/28/29	13.71		
		25.59	
Total credits			$1,627.37
Expenditures for 1929:			
Postage		$40.00	
Printing and stationery		101.00	
Expenses of Secretary (Rochester)		48.17	
Wistar Institute (Abstracts, Anat. Rec.)		72.86	
Local Committee on Arrangements (Rochester)		150.00	
Secretarial services		54.25	
Total expenditures			466.28
Balance on hand, December 31, 1929			$1,161.09

Deposited, in name of American Association of Anatomists, in Alexander Brown & Sons, Baltimore, Maryland.

Report of the Auditing Committee: Dr. Carl C. Speidel reported for the Auditing Committee as follows: "The undersigned Auditing Committee has examined the accounts of the Secretary-Treasurer for the year 1929. It finds receipts totaling those entered in the journal, expenditures in accordance with receipted bills, and the balance on hand agreeing with the bank statement of December 31, 1929."

[Signed] C. C. Speidel,
L. B. Arey.

On motion, duly seconded, the reports of the Treasurer and of the Auditing Committee were accepted and adopted.

Election of officers: Professor Eliot R. Clark, Chairman of the Committee on Nominations for 1930, placed before the Association the following nominations for office:

For President: Professor Herbert M. Evans, of the University of California.

For Vice-President: Professor Edgar Allen, of the University of Missouri.

For Secretary-Treasurer: Professor George W. Corner, of the University of Rochester.

For Members of the Executive Committee: Professor L. B. Arey, of Northwestern University, and Professor S. R. Detwiler, of Columbia University.

On motion, seconded and carried, the Secretary was instructed to cast a ballot for the election of the above named, with terms of office as specified in the Constitution of the Association.

New Members. The Secretary presented the following names recommended by the Executive Committee for election to membership in the Association:

CHARIPPER, HARRY A., B.S., M.S., Ph.D., Instructor in Comparative Anatomy, *Washington Square College, New York University, New York City.*

COREY, EDWARD LYMAN, A.B., Ph.D., Assistant Professor of Physiology, *University of Virginia, Charlottesville, Virginia.*

DAVENPORT, H. A., M.D., Instructor in Neurology, *Northwestern University Medical School, Chicago, Illinois.*

HATT, ROBERT T., Sc.B., M.A., Assistant Curator, Department of Mammology, *American Museum of Natural History, New York City.*

HEINBECKER, PETER, M.D., Assistant Professor of Surgery, *Washington University Medical School, St. Louis, Missouri.*

HELFF, O. M., Ph.D., Associate Professor of Biology, *New York University, New York City.*

HIRSCH, CHRISTIAN G., M.D., Research Fellow in Cytology, *Washington University School of Medicine, St. Louis, Missouri.*

HOADLEY, LEIGH, A.B., Ph.D., Associate Professor of Zoölogy, *Harvard University, Cambridge, Mass.*

KELLOGG, REMINGTON, Ph.D., Assistant Curator of Mammals, *U. S. National Museum, Washington, D. C.*

MACCALLUM, DANIEL BARTLETT, S.B., Ph.D., M.D., Instructor in Medicine, *University of Wisconsin, Madison, Wisconsin.*

MAINLAND, DONALD, M.B., Ch.B., Assistant Professor of Anatomy, *University of Manitoba, Winnipeg, Canada.*

SEVERINGHAUS, AURA E., B.S., M.A., Ph.D., Assistant Professor of Anatomy, *College of Physicians and Surgeons, Columbia University, New York City.*

STUNKARD, H. W., Ph.D., Professor of Biology, *New York University, New York City.*

THOMPSON, IAN MACLAREN, B.Sc., M.B., Ch.B., Associate Professor of Anatomy, *University of California, Berkeley, Calif.*

WEATHERFORD, HAROLD LORRAINE, A.B., A.M., Ph.D., Instructor in Anatomy, *Harvard Medical School, Boston, Mass.*

ZWEMER, RAYMOND L., A.B., Ph.D., Instructor in Anatomy, *College of Physicians and Surgeons, Columbia University, New York City.*

On motion, the Secretary was instructed to cast a ballot for all candidates proposed by the Executive Committee.

Dropped Members. The Secretary then announced the following names as dropped from the list of members on account of non-payment of dues for the past two years:

H. Arai, Sappora, Japan.
C. P. Chagas, Brazil, South America.
J. T. Scott, Denver, Colorado.
M. Takenouchi, Tokyo, Japan.
C. Tsai, Shanghai, China.

Death of Members. The Secretary then announced, on behalf of the Executive Committee, that during the past year the Association has suffered great loss in the death of the following active members:

Carl A. Hamann, Cleveland, Ohio.
John Sterling Kingsley, Berkeley, California.
William F. Mercer, Athens, Ohio.

Place of Next Meeting. It was announced that the Executive Committee had decided to hold the forty-seventh annual session of the Association at Northwestern University Medical School, Chicago, Illinois, on the three days immediately preceding Easter, 1931.

Dues for the Year 1930. The Executive Committee recommended that the dues for the year 1930 be set at $1.00 per member. This recommendation of the Executive Committee was adopted by the Association.

Payment to Local Committee. In accordance with the policy established by the Association at the forty-third session, it was moved and seconded that the Treasurer be authorized to pay the sum of $150.00 from the general funds of the Association to the Local Committee on Arrangements at the University of Virginia for the purpose of defraying the cost of entertainment of the Association during the present forty-sixth session.

Representatives of Association. On recommendation of the Executive Committee, the Association voted to make the following appointments of representatives: Professor Harvey E. Jordan, University of Virginia, to the Commission on Standardization of Biological Stains; Professor J. Parsons Schaeffer, Jefferson Medical College, to the Division of Medical Sciences of the National Research Council.

International Anatomical Congress. Professor Charles R. Stockard, Chairman of the Committee appointed by the Association, reported briefly on the arrangements made for the International Anatomical Congress, to be held in Amsterdam, August 4 to 9, 1930. It was announced that the Secretary of the Association would receive titles of papers to be presented before this Congress by members of the American Association of Anatomists.

Biological Abstracts. Dr. Stacy R. Guild, Editor of the section of Vertebrate Anatomy, and Dr. Carl G. Hartman, Editor of the section on Embryology, presented before the Association information relating to the preparation of abstracts for Biological Abstracts. On motion, it was voted to accept the statements made by the two editors as a report; the Association then voted unanimously to extend thanks to the two editors for the work done by them.

Resolution of Appreciation. Professor Eliot R. Clark presented the following resolution:

> *Resolved:* That the Association hereby expresses its appreciation of the superb services rendered the Association by Doctor Weed during his eight-year term of office as Secretary-Treasurer.

Unanimously voted.

On motion, the Business Session then adjourned.

SATURDAY, APRIL 19, 1930

12 noon. Concluding business session. A short business meeting followed the morning scientific sessions. The Secretary reported that there was no unfinished business.

Resolution of Appreciation. Professor Frederic T. Lewis presented the following resolution:

> *Resolved:* That, to the invariable attractions of a session of anatomists—the opportunity to hear of new progress in an ancient science, and to revisit old friends—there has been added at this meeting all the charm of Virginia in

springtime. We have held a meeting of unusual distinction, and have been entertained with traditional southern hospitality. In behalf of the Association of Anatomists, I move a vote of thanks to Doctors Jordan and Bean, and others representing the University of Virginia—our hostess on this occasion which will long be remembered.

Moved, seconded, and unanimously voted.

On motion, the Session adjourned.

LEWIS H. WEED,
Secretary of the Forty-sixth Session of
the American Association of Anatomists.

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de Aberle (Mrs.) Sophie B., A.B., A.M., Ph.D., Alexander Brown Coxe Fellow, *New Haven Hospital, New Haven, Conn.*

Adams, A. Elizabeth, A.B., A.M., Ph.D., Professor of Zoölogy, *Mount Holyoke College, South Hadley, Mass.*

Adams, L. A., A.B., A.M., Ph.D., Assistant Professor of Zoölogy, *University of Illinois, 207 Iowa St., Urbana, Ill.*

Addison, William Henry Fitzgerald, B.A., M.D., Professor of Normal Histology and Embryology, *School of Medicine, University of Pennsylvania, Philadelphia, Penna.*

Adelmann, Howard B., B.A., M.A., Ph.D., Assistant Professor of Histology and Embryology, *Cornell University, Stimson Hall, Ithaca, N. Y.*

Allen, Bennet Mills, Ph.D., Professor of Zoölogy, University of California (Southern Branch), *909 Micheltorena St., Los Angeles, Calif.*

Allen, Edgar, A.M., Ph.D. (Vice-Pres. '30–), Professor of Anatomy and Dean of the School of Medicine, *University of Missouri, Columbia, Mo.*

Allen, Ezra, A.M., Ph.D., Associate Editor, Biological Abstracts; Associate Professor of Histology and Embryology, *New York Homeopathic Medical School, York Ave. and Sixty-fourth St., New York City.*

Allen, William F., A.M., Ph.D., Professor of Anatomy, *University of Oregon Medical School, Portland, Oregon.*

Allis, Edward Phelps, Jr., C.E., M.D., LL.D., *Palais de Carnoles, Menton (A.M.), France.*

Andersen, Dorothy H., M.D., Assistant in Pathology, *Columbia University, 630 West 168th Street, New York City.*

Angulo y Gonzalez, Armando W., A.B., A.M., Ph.D., Fellow, *The Wistar Institute of Anatomy, Thirty-sixth St. and Woodland Ave., Philadelphia, Penna.*

Anson, Barry J., A.B., A.M., Ph.D., Associate in Anatomy, *Northwestern University Medical School, 303 East Chicago Ave., Chicago, Ill.*

Appleby, J. I., A.B., M.B., A.M., M.D., Physician and Surgeon, Company Surgeon, N. Y. C. & St. L. R. R. Co., *105½ East Main St., Bellevue, Ohio.*

Arataki, Minoru, *Director Arataki Hospital, Asahigawa, Japan.*

Arey, Leslie B., Ph.D. (Ex. Com. '30–), Robert Laughlin Rea Professor of Anatomy, and Chairman of the Division of Anatomy, *Northwestern University Medical School, 303 East Chicago Ave., Chicago, Ill.*

Armstrong, Philip Brownell, B.S., M.D., Instructor in Anatomy, *Department of Anatomy, Cornell Medical College, Twenty-eighth St. and First Ave., New York City.*

Atterbury, Ruth Rand, A.M., Ph.D., *144 Overlook Ave., Great Neck, L. I.*

Atwell, Wayne Jason, A.M., Ph.D., Professor of Anatomy, University of Buffalo Medical College, *24 High St., Buffalo, N. Y.*

Badertscher, Jacob A., Ph.D., Professor of Anatomy, Indiana University School of Medicine, *312 South Fess Ave., Bloomington, Ind.*

Bagley, Charles, Jr., M.D., *The Latrobe Apts., Baltimore, Md.*

Bailey, Percival, M.D., Ph.D., Associate Professor of Surgery, *University of Chicago, 950 East Fifty-ninth St., Chicago, Ill.*

Baitsell, George Alfred, B.S., M.A., Ph.D., Professor of Biology, Yale University, *Osborn Zoölogical Laboratory, New Haven, Conn.*

Baker, R. C., A.B., M.A., Ph.D., Associate Professor of Anatomy, *Department of Anatomy, Hamilton Hall, Ohio State University, Columbus, Ohio.*

Baldwin, Wesley Manning, A.B., A.M., M.D., Professor of Anatomy, *Albany Medical College, Albany, N. Y.*

Bardeen, Charles Russell, A.B., M.D. (Ex. Com. '06–'09, Vice-President '18–'20), Professor of Anatomy and Dean of Medical School, *University of Wisconsin, Science Hall, Madison, Wis.*

Barta, Edmund, M.D., Adjunct, Department of Anatomy, *Royal University Medical School, Budapest, Hungary.*

Bartelmez, George W., Ph.D., Associate Professor of Anatomy, *University of Chicago, Chicago, Ill.*

Bartsch, Paul, B.S., M.S., Ph.D., Curator of Mollusks and Cenozoic Invertebrates, *U. S. National Museum, Washington, D. C.*

Bascom, Kellogg F., B.A., M.S., Ph.D., M.D., *2940 Oakdale Ave., St. Louis Park, Minn.*

Bast, T. H., B.A., Ph.D., Associate Professor of Anatomy, *University of Wisconsin, 308 Science Hall, Madison, Wis.*

Batson, O. V., A.M., M.D., Professor of Anatomy, *Department of Anatomy, University of Pennsylvania, Philadelphia, Penna.*

Baumgartner, William J., A.B., A.M., Ph.D., Associate Professor of Zoölogy, University of Kansas, *1209 Ohio St., Lawrence, Kans.*

Bayon, Henry, B.A., M.D., Emeritus Professor of Applied Anatomy, Tulane University, *2212 Napoleon Ave., New Orleans, La.*

Bean, Robert Bennett, B.S., M.D., Professor of Anatomy, University of Virginia, *5 Wayside Place, University, Va.*

Beattie, John, B.Sc., M.Sc., M.B., M.D., Associate Professor of Anatomy, *Department of Anatomy, McGill University, Montreal, Canada.*

Begg, Alexander S., B.S., M.D., Professor of Anatomy and Dean, Boston University School of Medicine, *80 East Concord St., Boston, Mass.*

Bensley, Robert Russell, A.B., M.B., Sc.D. (Second Vice-Pres. '06–'07, Ex. Com. '08–'12, President '18–'20), Professor of Anatomy, Director of Hull Laboratory of Anatomy, *University of Chicago, Chicago, Ill.*

Best, Russell R., B.Sc., M.D., Assistant Professor of Anatomy, *Department of Anatomy, University of Nebraska, College of Medicine, 1036 Medical Arts Building, Omaha, Nebraska.*

Bevan, Arthur Dean, M.D. (Ex. Com. '96–'98), Professor of Surgery, University of Chicago, *122 South Michigan Blvd., Chicago, Ill.*

Bigelow, Robert P., S.B., Ph.D., Professor of Zoölogy and Parasitology, *Massachusetts Institute of Technology, Cambridge 39, Mass.*

Bishop, Katherine Scott, A.B., M.D., Research Associate, Hooper Research Institute, San Francisco, *1508 La Loma Ave., Berkeley, Calif.*

Black, Davidson, B.A., M.B., Professor of Anatomy, *Peking Union Medical College, Peking, China.*

Blair, Vilray Papin, A.M., M.D., Associate in Clinical Surgery, Washington University School of Medicine, *Metropolitan Building, St. Louis, Mo.*

Blaisdell, Frank Ellsworth, Sr., M.D., Emeritus Professor of Surgery, Medical Department of Stanford University, *Lane Medical Library Building, corner Webster and Sacramento Sts., San Francisco, Calif.*

Blake, J. A., A.B., Ph.B., M.A., M.D., F.A.C.S., Consulting Surgeon, St. Luke's, Roosevelt, Orthopedic, Lincoln, Dobbs Ferry, St. John's (Yonkers) Hospitals, *116 East Fifty-third St., New York City.*

Blincoe, Homer, A.B., M.S., M.D., Professor of Gross Anatomy, *Emory University, Emory, Georgia.*

Bloom, William, A.B., M.D., Assistant Professor of Anatomy, *Department of Anatomy, University of Chicago, Chicago, Ill.*

Boyd, Edith, M.D., Assistant Professor, *Institute of Child Welfare, University of Minnesota, Minneapolis, Minn.*

Boyden, Edward Allen, A.M., Ph.D. Ex. Com. '28–), Professor of Anatomy, *University of Alabama, Tuscaloosa, Alabama.*

Bremer, John Lewis, A.B., M.D. (Ex. Com. '15–'18), Associate Professor of Histology, *Harvard Medical School, Boston, Mass.*

Browning, William, Ph.B., M.D., Emeritus Professor of Neurology, Long Island College Medical School, *54 Lefferts Place, Brooklyn, N. Y.*

Bryce, Thomas H., M.A., M.D., F.R.S., Professor of Anatomy, University of Glasgow, *No. 2, The University, Glasgow, Scotland.*

Bullard, H. Hays, A.M., Ph.D., M.D., Professor of Pathology, *University of Western Ontario Medical School, London, Ontario, Canada.*

Bunting, Charles Henry, B.S., M.D., Professor of Pathology, *University of Wisconsin, Madison, Wis.*

Burns, B. I., M.D., Dean and Professor of Anatomy, *University of Utah, School of Medicine, Salt Lake City, Utah.*

Burns, Robert K., Jr., A.B., Ph.D., Assistant Professor of Anatomy, *Department of Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Burr, Harold Saxton, Ph.D., Associate Professor of Anatomy, School of Medicine, Yale University, *333 Cedar St., New Haven, Conn.*

Burrows, Montrose T., A.B., M.D., Physician and Surgeon, Chairman Cancer Clinic, Pasadena Hospital, Consultant Pathologist Los Angeles County General Hospital, *94 North Madison Ave., Pasadena, Calif.*

Butcher, Earl O., A.M., Associate Professor of Biology, *Hamilton College, Griffin Road, College Hill, Clinton, N. Y.*

Butler, Elmer G., A.B., Ph.D., Assistant Professor of Biology, *Laboratory of Comparative Anatomy, Princeton University, Princeton, N. J.*

Calkins, Leroy A., B.S., M.S., M.B., M.D., Ph.D., Professor of Obstetrics and Gynecology, University of Virginia Medical School, *Box 238, University, Va.*

Cameron, Angus L., A.B., M.S., M.D., Ph.D., *128 South Main St., Minot, N. Dak.*

Cameron, John, M.D., D.Sc., F.R.S.E., Professor of Anatomy, *Dalhousie Medical College, Halifax, Nova Scotia.*

Cardwell, John C., M.D., Professor of Physiology, Long Island College Hospital, *Polhemus Memorial Clinic, Brooklyn, N. Y.*

Carey, Eben J., M.S., Sc.D., Acting Dean of Students, Professor of Anatomy, Marquette University School of Medicine, *638 Fourth St., Milwaukee, Wis.*

Carver, Gail L., A.B., A.M., Professor of Biology, Mercer University, *613 Orange St., Macon, Ga.*

Casamajor, Louis, A.M., M.D., Professor of Neurology, *Columbia University, 630 West 168th St., New York City.*

Chambers, Robert, Jr., A.M., Ph.D., Research Professor of Biology and Chairman of the Department of Biology, *Washington Square College, New York University, New York City.*

Chang, Chun, M.D., Associate Professor of Anatomy, *National Central University, College of Medicine, Woosung, Shanghai, China.*

Charipper, Harry A., B.S., M.S., Ph.D., Instructor in Comparative Anatomy, *Washington Square College, New York University, New York City.*

Charlton, Harry Hayward, A.B., A.M., Ph.D., Associate Professor of Anatomy, *Department of Anatomy, University of Missouri, Columbia, Mo.*

Chase, Samuel Wood, A.B., A.M., Ph.D., Associate Professor of Histology and Embryology, *Western Reserve University School of Medicine, 2109 Adelbert Road, Cleveland, Ohio.*

Chidester, Floyd E., A.M., Ph.D., Professor of Zoölogy, *University of West Virginia, Morgantown, W. Va.*

Child, Charles Manning, M.S., Ph.D., Professor of Zoölogy, *Zoölogical Laboratory, University of Chicago, Chicago, Ill.*

Chillingworth, Felix P., M.D., Professor of Physiology and Experimental Pharmacology, Tufts Medical College, *416 Huntington Ave., Boston, Mass.*

Chouke, Kehar Singh, M.D., Assistant Professor of Anatomy, *Colorado University Medical School, 4200 East Ninth Ave., Denver, Colo.*

Clark, Eleanor Linton, A.M., Voluntary Research Worker, Department of Anatomy, *Medical Department, University of Pennsylvania, Philadelphia, Pa.*

Clark, Eliot R., A.B., M.D. (Ex. Com. '16–'19), Professor of Anatomy and Director of Department of Anatomy, University of Pennsylvania, *301 St. Mark's Square, Philadelphia, Pa.*

Clark, Sam L., Ph.D., Assistant Professor of Anatomy, *Northwestern Medical School, Chicago, Ill.*

Coe, Wesley R., Ph.D., *175 Bishop St., New Haven, Conn.*

Coghill, George E., A.B., M.S., Ph.D. (Ex. Com. '27–), Member of The Wistar Institute, *The Wistar Institute of Anatomy and Biology, Woodland Ave. and Thirty-sixth St., Philadelphia, Pa.*

Cohn, Alfred E., A.B., M.D., Member, *Rockefeller Institute for Medical Research, New York City, N. Y.*

Collins, Henry Homer, Ph.D., Professor of Zoölogy, *University of Pittsburgh, Pittsburgh, Pa.*

Coman, Francis Dana, A.B., A.M., M.D., Assistant in Surgery, Johns Hopkins University, *Johns Hopkins Hospital, Baltimore, Md.*

Conant, William Merritt, A.B., M.D., Surgeon, *636 Beacon St., Boston, Mass.*

Conel, Jesse LeRoy, A.M., Ph.D., Professor of Anatomy, Boston University School of Medicine, *80 E. Concord St., Boston, Mass.*

Congdon, Edgar Davidson, Ph.D., Professor of Anatomy, *Chulalongkorn University Medical School, Bangkok, Siam.*

Conklin, Edwin Grant, A.M., Ph.D., Sc.D., LL.D., Professor of Biology, Princeton University, *139 Broadmead Ave., Princeton, N. J.*

Copenhaver, Wilfred M., A.B., Ph.D., Assistant Professor of Anatomy, *College of Physicians and Surgeons, Columbia University, 630 West 168th St., New York City.*

Corey, Edward Lyman, A.B., Ph.D., Assistant Professor of Physiology, *University of Virginia, Charlottesville, Virginia.*

Corner, George W., A.B., M.D. (Ex. Com. '26–'30; Sec.-Treas. '30–), Professor of Anatomy, *University of Rochester, Crittenden Boulevard, Rochester, N. Y.*

Corning, H. K., M.D., Professor of Anatomy, *University of Bâle, Anatomische Anstalt, Bâle, Switzerland.*

Covell, W. P., Ph.D., Assistant, *Rockefeller Institute for Medical Research, New York City.*

Cowdry, Edmund V., Ph.D. (Ex. Com. '26–'30), Professor of Cytology, *Washington University Medical School, St. Louis, Missouri.*

Craigie, E. Horne, B.A., Ph.D., Assistant Professor of Comparative Anatomy and Neurology, *Department of Biology, University of Toronto, Toronto, 5, Canada.*

Crile, George W., A.M., M.D., LL.D., F.A.C.S., Professor of Surgery, Western Reserve University, *Cleveland Clinic, 93rd and Euclid, Cleveland, Ohio.*

Crosby, Elizabeth Caroline, M.S., Ph.D., Associate Professor of Anatomy, University of Michigan, *201 N. Thayer St., Ann Arbor, Mich.*

Cuajunco, Fidel, M.D., Assistant Professor of Anatomy, College of Medicine, University of the Philippines, *620 Colorado St., Manila, P. I.*

Cullen, Thomas S., M.B., D.Sc., Professor of Clinical Gynecology, Johns Hopkins University, *20 E. Eager St., Baltimore, Md.*

Cummins, Harold, A.B., Ph.D., Associate Professor of Anatomy, *Tulane University Medical School, Department of Anatomy, Sta. 20, New Orleans, La.*

Cunningham, Robert S., A.M., M.D., Professor of Anatomy, *Vanderbilt School of Medicine, Nashville, Tenn.*

Curtis, George M., A.M., Ph.D., M.D., Associate Professor of Surgery, *Billings Memorial Hospital, University of Chicago, Chicago, Ill.*

Dahlgren, Ulric, A.B., M.S., Professor of Biology, Princeton University, *204 Guyot Hall, Princeton, N. J.*

Danforth, Charles Haskell, A.M., Ph.D. (Ex. Com. '27–), Professor of Anatomy, Leland Stanford University, *Stanford University, Calif.*

Darrach, William, A.B., A.M., M.D., Professor of Clinical Surgery and Dean of Faculty of Medicine, College of Physicians and Surgeons, Columbia University, *632 West 168th St., New York City.*

Dart, Raymond A., M.B., Ch.M., M.Sc., Professor of Anatomy and Dean of Faculty of Medicine, *University of the Witwatersrande, Johannesburg, Transvaal, South Africa.*

Davenport, H. A., M.D., Instructor in Neurology, *Northwestern University Medical School, Chicago, Illinois.*

Davis, Carl L., M.D., Professor of Anatomy, *Medical Department, University of Maryland, Baltimore, Md.*

Davis, Warren B., M.D., F.A.C.S., Instructor in Surgery, Jefferson Medical College; Oto-rhino-laryngologist, St. Agnes Hospital; Maxillo-facial Surgeon, *135 South Eighteenth St., Philadelphia, Pa.*

Dawson, Alden B., A.B., Ph.D., Associate Professor of Zoölogy, Zoölogical Laboratory, *Harvard University, Cambridge, Mass.*

De Carlo, John, M.D., Demonstrator of Topographic and Applied Anatomy, Jefferson Medical College, *1629 S. Broad St., Philadelphia, Pa.*

DeGaris, Charles F., A.B., M.D., Associate in Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*

Detwiler, Samuel Randall, A.M., Ph.D., Ph.B. (Ex. Com. '30–), Professor of Anatomy, Department of Anatomy, *College of Physicians and Surgeons, Columbia University, 630 West 168th St., New York City.*

Dixon, A. Francis, M.B., Sc.D., University Professor of Anatomy, Trinity College, *42 Elgin Road, Dublin, Ireland.*

Doan, Charles A., A.B., M.D., Associate, *Rockefeller Institute for Medical Research, Sixty-sixth St. and Avenue A, New York City.*

Dodds, Gideon S., A.M., Ph.D., Professor of Histology and Embryology, *West Virginia University Medical School, Morgantown, W. Va.*

Dodson, John Milton, A.M., M.D., Professor (Emeritus) of Medicine, *Rush Medical College, 5707 Blackston Ave., Chicago, Ill.*

Donaldson, Henry Herbert, Ph.D., D.Sc. (Ex. Com. '09–'13, Pres. '16–'17), Member of The Wistar Institute, *The Wistar Institute of Anatomy and Biology, Woodland Ave. and Thirty-sixth St., Philadelphia, Pa.*

Donaldson, John C., Ph.B., M.D., Associate Professor of Anatomy, *School of Medicine, University of Pittsburgh, Pittsburgh, Pa.*

Downey, Hal, A.M., Ph.D., Professor of Histology, *Department of Animal Biology, University of Minnesota, Minneapolis, Minn.*

Downs, William Gershom, Jr., A.B., A.M., D.D.S., Assistant Professor of Pathology, *Yale Medical School, 310 Cedar St., New Haven, Conn.*

Duesberg, Jules, M.D., Professor of Anatomy, *University of Liege, 22 quai Mativa, Liege, Belgium.*

Duncan, Donald, A.B., A.M., Ph.D., Assistant Professor of Anatomy, Department of Anatomy, *University of Utah, Salt Lake City, Utah.*

Dunn, H. L., M.A., B.A., M.B., Ph.D., M.D., Associate Professor of Biometry and Statistical Medicine, *University of Minnesota, Mayo Clinic, Rochester, Minnesota.*

Eccles, Robert G., M.D., Phar.D., *681 Tenth St., Brooklyn, N. Y.*

Edwards, J. Graham, Ph.D., Assistant Professor of Anatomy, *Department of Anatomy, University of Buffalo School of Medicine, 24 High St., Buffalo, N. Y.*

Elwyn, Adolph, A.M., Assistant Professor of Neurology, *Columbia University, 630 West 168th St., New York City.*

Engle, Earl Theron, A.B., A.M., Ph.D., Associate Professor of Anatomy, *College of Physicians and Surgeons, Columbia University, 632 West 168th St., New York City.*

Esaki, Shiro, Assistant Professor of Anatomy, *Keio-Gigyuku University, Yotsuya, Tokyo, Japan.*

Essick, Charles Rhein, B.A., M.D., *439 Oley St., Reading, Pa.*

Evans, Herbert McLean, B.S., M.D., (Ex. Com. '19–'22; Pres. '30–), Professor of Anatomy and Research Associate in Experimental Biology, *University of California, Berkeley, Calif.*

EVANS, THOMAS HORACE, M.D., Associate Professor of Anatomy, Long Island College Hospital, *Room 83, Polhemus Memorial, Brooklyn, N. Y.*

FARIS, HERVEY S., A.B., A.M., Ph.D., M.D., Practicing Physician and Surgeon, *3770 Twelfth St., Riverside, Calif.*

FERRIS, HARRY BURR, A.B., M.D., Hunt Professor of Anatomy, Medical Department, Yale University, *395 St. Ronan St., New Haven, Conn.*

FETTEROLF, GEORGE, A.B., M.D., Sc.D., Professor of Oto-laryngology, University of Pennsylvania, *2010 Spruce St., Philadelphia, Pa.*

FISCHELIS, PHILIP, M.D., Professor of Histology and General Pathology, Philadelphia Dental College of Temple University, *2132 N. 19th St., Philadelphia, Pa.*

FLORENCE, LAURA, B.Sc., M.A., Ph.D., Associate Professor of Bacteriology, *New York Homeopathic Medical College, 445 E. Sixty-fifth St., New York City.*

FOLEY, JAMES OWEN, M.S., Ph.D., Assistant Professor of Microscopic Anatomy, *Tulane University of Louisiana, New Orleans, La.*

FORMAN, JONATHAN, A.B., M.D., Director of Pathological Laboratories, Grant Hospital, *394 East Town St., Columbus, Ohio.*

FRASSETTO, FABIO, Ph.D., Director Anthropological Institute, *University of Bologna, Bologna, Italy.*

FRAZER, JOHN ERNEST, M.D., F.R.C.S., Professor of Anatomy, University of London, *St. Mary's Hospital Medical School, London, W., England.*

FRENCH, H. E., M.S., M.D., Professor of Anatomy and Dean of the School of Medicine, *University of North Dakota, Grand Forks, North Dakota.*

GAGE, SIMON HENRY, B.S. (Ex. Com. '06–'11), Professor of Histology and Embryology, Emeritus, *Stimson Hall, Cornell University, Ithaca, N. Y.*

GALLAUDET, BERN BUDD, A.B., A.M., M.D., Associate Professor of Anatomy, Columbia University, Consulting Surgeon, Bellevue Hospital, *601 West 168th St., New York City.*

GAMBLE, DEAN L., B.S., Ph.D., Head Zoölogical Department, General Biological Supply House, *761 East Sixty-ninth Place, Chicago, Ill.*

GARCIA, ARTURO, A.B., M.D., Professor of Anatomy, College of Medicine, University of the Philippines, *619 Colorado St., Manila, Philippine Islands.*

GEIST, FREDERICK D., M.D., Assistant Professor of Anatomy, *University of Wisconsin, Science Hall, Madison, Wis.*

GEORGE, WESLEY CRITZ, A.B., A.M., Ph.D., Professor of Histology and Embryology, *University of North Carolina Medical School, Chapel Hill, N. C.*

GILLASPIE, C., M.D., F.A.C.S., Associate Professor of Anatomy, *University of Colorado, Boulder, Colo.*

GLOBUS, J. H., B.S., M.D., Assistant Professor of Neuro-anatomy, New York University and Bellevue Medical College, *1075 Park Ave., New York City.*

GOSS, CHARLES MAYO, A.B., M.D., Instructor in Anatomy, *Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York City.*

GOULD, HARLEY NATHAN, A.B., A.M., Ph.D., Professor of Biology, *Newcomb College, Tulane University, New Orleans, La.*

GRANT, J. C. BOILEAU, M.D., Ch.B., F.R.C.S., Professor of Anatomy, *University of Manitoba Medical College, Winnipeg, Canada.*

GRAVES, WILLIAM W., M.D., Professor and Director, Department of Neuropsychiatry, *St. Louis University School of Medicine, St. Louis, Mo.*

GREENE, CHARLES W., A.M., Ph.D., Professor of Physiology and Pharmacology, University of Missouri, *814 Virginia Ave., Columbia, Mo.*

GREENE, WALTER FARRAR, A.B., Ph.D., Associate Professor of Anatomy, *Syracuse College of Medicine, 309 South McBride St., Syracuse, N. Y.*

GREENHILL, J. P., B.S., M.D., F.A.C.S., Attending Obstetrician, The Chicago Lying-in-Hospital, Associate in Obstetrics, Northwestern Medical School, Attending Gynecologist, Cook County Hospital, *426 East Fifty-first St., Chicago, Ill.*

GREENMAN, MILTON J., Ph.B., M.D., Sc.D., Director of *The Wistar Institute of Anatomy and Biology, 36th St. and Woodland Ave., Philadelphia, Pa.*

GREGORY, WILLIAM KING, A.B., A.M., Ph.D., Professor of Vertebrate Palaeontology, Columbia; Associate in Vertebrate Palaeontology, American Museum of Natural History, and Curator of Comparative Anatomy, *American Museum of Natural History, 136 West 77th St., New York City.*

GUDERNATSCH, J. F., Ph.D., Visiting Professor, Department of Biology, *New York University, Washington Square East, New York City.*

GUILD, STACY R., A.M., Ph.D., Lecturer in Otology and Director of Otological Research Laboratory, Johns Hopkins University, *Johns Hopkins Hospital, Baltimore, Md.*

GURDJIAN, ELISHA STEPHENS, A.B., M.S., M.D., Ph.D., Assistant Resident in Surgery, *Rochester General Hospital, Rochester, N. Y.*

GUTTMACHER, ALAN F., A.B., M.D., Clinical Instructor in Obstetrics, Johns Hopkins University, *824 Park Ave., Baltimore, Md.*

GUYER, MICHAEL F., Ph.D., Professor of Zoölogy, *University of Wisconsin, Madison, Wis.*

HALL, E. RAYMOND, Ph.D., Curator of Mammals, *California Museum of Vertebrate Zoölogy, University of California, Berkeley, Calif.*

HALPERT, BÉLA, M.D., Assistant Professor of Pathology, *Department of Pathology, University of Chicago, Chicago, Ill.*

HANAN, ERNEST B., A.B., A.M., M.D., Associate in Pharmacology, *University of Buffalo, Buffalo, N. Y.*

HANSON, FRANK BLAIR, A.B., A.M., Ph.D., Professor of Zoölogy, *Washington University, St. Louis, Mo.*

HARDESTY, IRVING, A.B., Ph.D., Sc.D. (Ex. Com. '10 and '12–'15, '22–'23), Professor of Anatomy and Head of Department of Anatomy, School of Medicine, *Tulane University, P. O. Station 20, New Orleans, La.*

HARE, EARL R., A.B., M.D., F.A.C.S., *730 LaSalle Building, Minneapolis, Minn.*

HARGITT, GEORGE T., A.B., A.M., Ph.D., Professor of Zoölogy, *Lyman Hall, Syracuse University, Syracuse, N. Y.*

HARRIS, HENRY A., M.B., B.S., F.Z.S., M.R.C.S., L.R.C.P., D.Sc., Assistant Professor of Anatomy and Assistant to the Medical Unit, *Institute of Anatomy, University College, Gower St., London, W.C.1, England.*

HARRISON, ROSS GRANVILLE, Ph.D., M.D., Sc.D. (Pres. '12–'14), Sterling Professor of Biology, *Osborn Zoölogical Laboratory, Yale University, New Haven, Conn.*

HARTMAN, CARL G., Ph.D., Research Associate, *Carnegie Laboratory of Embryology, Johns Hopkins Medical School, Baltimore, Md.*

HARVEY, BASIL COLEMAN HYATT, A.B., M.B., Professor of Anatomy and Dean of Medical Students, University of Chicago, *Department of Anatomy, University of Chicago, Chicago, Ill.*

HARWELL, CHAS. W., A.B., M.D., Assistant Professor of Anatomy, *Emory University, Emory, Ga.*

HATAI, SHINKISHI, Ph.D., Professor of Neurology, *c/o Tohoku Imperial University, Sendai, Japan.*

HATT, ROBERT T., Sc.B., M.A., Assistant Curator, Department of Mammology, *American Museum of Natural History, New York City.*

HAUSMAN, LOUIS, A.B., M.D., Associate in Neuro-anatomy, *1175 Park Ave., New York City.*

HAZEN, CHARLES MORSE, A.M., M.D., *Professional Building, Fifth and Franklin Sts., Richmond, Va.*

HEINBECKER, PETER, M.D., Assistant Professor of Surgery, *Washington University Medical School, St. Louis, Missouri.*

HEISLER, JOHN C., M.D., Professor of Anatomy, University of Pennsylvania, *3829 Walnut St., Philadelphia, Pa.*

HELDT, THOMAS JOHANES, A.B., A.M., M.D., Physician in Charge of Neuropsychiatric Division, *Henry Ford Hospital, Detroit, Mich.*

HELFF, O. M., Ph.D., Associate Professor of Biology, *New York University, New York City.*

HEMLER, WILLIAM FRANCIS, M.D., Professor of Roentgenology, Georgetown University, *1230 Seventeenth St., N. W., Washington, D. C.*

HERRICK, CHARLES JUDSON, Ph.D. (Ex. Com. '13–'17), Professor of Neurology, University of Chicago, *Department of Anatomy, University of Chicago, Chicago, Ill.*

HERTZLER, ARTHUR E., B.S., A.M., Ph.D., M.D., LL.D., F.A.C.S., Professor of Surgery, *University of Kansas, Halstead, Kansas.*

HETHERINGTON, DUNCAN CHARTERIS, Ph.D., M.D., Instructor in Anatomy, *Vanderbilt University Medical School, Nashville, Tenn.*

HEUSER, CHESTER H., A.M., Ph.D., Research Associate, Department of Embryology, Carnegie Institution, *Johns Hopkins Medical School, Baltimore, Md.*

HEWSON, ADDINELL, A.B., A.M., M.D., F.A.C.S., Emeritus Professor of Anatomy, Graduate School of Medicine, University of Pennsylvania, Professor of Anatomy and Histology, Temple University, *257 South Sixteenth St., Philadelphia, Pa.*

HIGGINS, GEORGE M., A.M., Ph.D., Associate in Experimental Surgery, *University of Minnesota, Mayo Foundation, Rochester, Minn.*

HILL, EBEN CLAYTON, A.B., M.D., Lecturer in Roentgenological Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*

HILL, HOWARD, M.D., Chief of Staff, St. Joseph Hospital; Surgical Staff, Kansas City General Hospital, *734 Argyle Building, Kansas City, Mo.*

HILL, JAMES PETER, D.Sc., F.R.S., Professor of Embryology, University of London, *University College, Gower St., London., W. C. 1, England.*

HILTON, WILLIAM A., B.S., Ph.D., Professor of Zoölogy, Pomona College, Director *Laguna Marine Laboratory, Claremont, Calif.*

Hines, Marion, A.B., Ph.D., Associate in Anatomy, Department of Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*

Hinsey, Joseph C., Ph.D., Associate Professor of Neuro-anatomy, *Institute of Neurology, Northwestern University School of Medicine, 303 E. Chicago Ave., Chicago, Ill.*

Hirsch, Christian G., M.D., Research Fellow in Cytology, *Washington University School of Medicine, St. Louis, Missouri.*

Hoadley, Leigh, A.B., Ph.D., Associate Professor of Zoölogy, *Harvard University, Cambridge, Mass.*

Hogg, Ira D., B.A., M.A., Ph.D., Assistant Professor of Anatomy, *Department of Anatomy, University of Pittsburgh School of Medicine, Pittsburgh, Pa.*

Hogue, Mary J., Ph.D., Instructor in Normal Histology, *University of Pennsylvania Medical School, Philadelphia, Pa.*

Holmgren, Nils, M.D., Professor of Zoölogy, *University of Stockholm, Sweden.*

Holt, Caroline M., A.B., A.M., Ph.D., Associate Professor of Biology and Public Health, Simmons College, *101 St. Paul St., Brookline, Mass.*

Hooker, Davenport, M.A., Ph.D. (Ex. Com. '22–'25), Professor of Anatomy, *School of Medicine, University of Pittsburgh, Oakland Station, Pittsburgh, Pa.*

Hopkins, Grant Sherman, Sc.D., D.V.M., Professor Comparative Veterinary Anatomy, *Cornell University, Ithaca, N. Y.*

Hoskins, Margaret Morris, Ph.D., Associate Professor of Microscopic Anatomy, *New York University Dental College, 209 East Twenty-third St., New York City.*

Howell, A. Brazier, A.B., Lecturer in Comparative Anatomy, *Department of Anatomy, Johns Hopkins Medical School, Baltimore, Md.*

Howland, Ruth B., Ph.D., Associate Professor of Biology, Washington Square College, *New York University, Washington Square East, New York City.*

Hoy, Wm. E., Jr., A.B., Ph.D., Professor of Biology and Head of the Department of Biology, University of South Carolina, *1401 Hagood Ave., Columbia, S. C.*

Hrdlička, Aleš, Ph.D., M.D., Curator, Division of Physical Anthropology, *United States National Museum, Washington, D. C.*

Huber, Ernst, Ph.D., M.D., Associate Professor of Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*

Huber, G. Carl, M.D. (Second Vice-Pres. '00–'01, Secretary-Treasurer '02–'14, Pres. '14–'16, Ex. Com. '20–'23), Professor of Anatomy and Director of the Anatomical Laboratories, University of Michigan, *1330 Hill St., Ann Arbor, Mich.*

Hughson, Walter, S.B., M.D., Associate in Clinical Surgery, Assistant Visiting Surgeon, Johns Hopkins Hospital, *1411 Eutaw Place, Baltimore, Md.*

Humphrey, Rufus R., A.B., A.M., Ph.D., Associate Professor of Anatomy, School of Medicine, University of Buffalo, *24 High St., Buffalo, N. Y.*

Hunter, Richard Henry, Ph.D., M.D., M.Ch., F.Z.S., Senior Lecturer in Anatomy, *Department of Anatomy, Queen's University, Belfast, Ireland.*

Hunter, Oscar B., A.B., A.M., M.D., Assistant Dean and Professor of Pathology and Bacteriology, *George Washington Medical School, 1335 H St., N.W., Washington, D. C.*

Ingalls, N. William, B.S., M.D., Associate Professor of Anatomy, School of Medicine, Western Reserve University, *2109 Adelbert Road, Cleveland, Ohio.*

INGVAR, SVEN, M.D., Docent in Neurology, *University of Lund, Lund, Sweden.*

JACKSON, CLARENCE M., M.S., M.D. (Ex. Com. '10–'14, '23–'28, Vice-Pres. '16–'17, Pres. '22–'23), Professor and Director of the Department of Anatomy, *Institute of Anatomy, University of Minnesota, Minneapolis, Minn.*

JENKINS, GEORGE B., M.D., Professor of Anatomy, *George Washington University Medical School, Washington, D. C.*

JOB, THESLE T., A.B., M.S., Ph.D., Professor of Anatomy, *Loyola University School of Medicine, 706 South Lincoln St., Chicago, Ill.*

JOHNSON, CHARLES EUGENE, A.M., Ph.D., Head of Department and Professor of Zoölogy and Director of the Roosevelt Wild Life Forest Experiment Station, *Department of Forest Zoölogy, State College of Forestry, Syracuse, N. Y.*

JOHNSON, FRANKLIN P., A.M., Ph.D., M.D., Instructor in Urology, University of Oregon Medical School, *303 Medical Arts Building, Portland, Ore.*

JOHNSON, SYDNEY E., Ph.D., M.D., Professor of Gross Anatomy, School of Medicine, University of Louisville, *101 West Chestnut St., Louisville, Ky.*

JOHNSTON, JOHN B., Ph.D., Dean of the College of Science, Literature and the Arts, Professor of Comparative Neurology, *University of Minnesota, Minneapolis, Minn.*

JOHNSTON, THOMAS BAILLIE, M.B., Ch.B., Professor of Anatomy, University of London, *Guy's Hospital Medical School, London, S. E. 1, England.*

JOHNSTONE, PAUL N., A.B., M.D., *601 Lathrop Building, Kansas City, Mo.*

JORDAN, HARVEY ERNEST, A.M., Ph.D. (Ex. Com. '18–'21), Professor of Histology and Embryology, *Department of Medicine, University of Virginia, Charlottesville, Va.*

KAAN, HELEN WHARTON, Ph.D., Visiting Assistant Professor, Department of Zoölogy and Physiology, *Wellesley College, Wellesley, Mass.*

KAMPMEIER, OTTO FREDERICK, A.B., Ph.D., M.D., Professor of Anatomy, *College of Medicine, University of Illinois, 1817 West Polk Street, Chicago, Ill.*

KAPPERS, CORNELIUS URBO ARIËNS, M.D., Director of the Central Institute for Brain Research of Holland, *Mauritskade 61, Amsterdam, Holland.*

KEEGAN, JOHN J., A.M., M.D., Dean, Professor of Neurological Surgery, *University of Nebraska, College of Medicine, Forty-second St. and Dewey Ave., Omaha, Neb.*

KEILLER, WILLIAM, L.R.C.P. and F.R.C.S. Ed. (Second Vice-Pres. '98–'99), Professor of Anatomy, Medical Department, University of Texas, *State Medical College, Galveston, Texas.*

KEITH, ARTHUR, M.D., LL.D., F.R.C.S., F.R.S., Hunterian Professor of Anatomy, *Royal College of Surgeons, Lincoln's Inn Fields, London, W. C. 2, England.*

KELLOGG, HOWARD BUTTERS, B.S., M.S., Ph.D., M.B., *5738 Stony Island Ave., Chicago, Ill.*

KELLOGG, REMINGTON, Ph.D., Assistant Curator of Mammals, *U. S. National Museum, Washington, D. C.*

KELLY, G. LOMBARD, A.B., B.S., M.D., Professor of Anatomy, *University of Georgia, Medical Department, Augusta, Georgia.*

KERNAN, JOHN D., JR., A.B., M.D., Associate Professor of Oto-Laryngology, Columbia College of Physicians and Surgeons, *120 East Seventy-fifth St., New York City.*

Kerr, Abram T., B.S., M.D. (Ex. Com. '10–'14), Professor of Anatomy and Secretary of Cornell University Medical School, *Cornell University Medical College, Ithaca, N. Y.*

Key, J. Albert, B.S., M.D., Director of Research, *Shriner's Hospital for Crippled Children, St. Louis, Mo.*

Kindred, James Ernest, A.B., A.M., Ph.D., Associate Professor of Histology and Embryology, University of Virginia, *R.F.D. No. 4, Charlottesville, Va.*

King, Helen Dean, A.M., Ph.D., Member of The Wistar Institute (Experimental Zoölogy), *The Wistar Institute of Anatomy, 36th St. and Woodland Ave., Philadelphia, Pa.*

King, Jessie L., Ph.D., Professor of Physiology, *Goucher College, Baltimore, Md.*

Kingery, Hugh McMillan, A.B., A.M., Ph.D., Associate Professor of Anatomy, *School of Medicine, University of Colorado, Denver, Col.*

Kingsbury, Benjamin F., A.B., Ph.D., M.D. (Ex. Com. '22–'25), Professor of Histology and Embryology, Cornell University, *2 South Ave., Ithaca, N. Y.*

Kirkham, William Barri, Ph.D., Professor of Biology and Dean of Freshmen, *Springfield College, 275 Maple St., Springfield, Mass.*

Knouff, R. A., B.A., M.A., Ph.D., Associate Professor of Anatomy, *Department of Anatomy, Ohio State University, Columbus, Ohio.*

Knower, Henry McE., A.B., Ph.D. (Ex. Com. '11–'15, '24–'26), *Wistar Institute of Anatomy, Philadelphia, Pa.*

Koch, John C., B.S., M.D., Junior Physician, Harper Hospital, *265 Euclid Ave., East, Detroit, Mich.*

Kofoid, Charles Atwood, A.B., Ph.D., Sc.D., Professor of Zoölogy and Chairman of Department, University of California, *Department of Zoölogy, University of California, Berkeley, Calif.*

Kornhauser, S. I., M.A., Ph.D., Professor of Anatomy and Executive Secretary, *University of Louisville, Medical School, Louisville, Ky.*

Krause, Allen Kramer, A.B., A.M., M.D., President and Director, *The Desert Sanatorium, Tucson, Arizona.*

Kudo, Tokuyasu, M.D., Professor of Anatomy and Histology, *Niigata Medical College, Niigata City, Echigo, Japan.*

Kunitomo, Kanae, M.D., Professor of Anatomy, *Anatomical Institute, Nagasaki Medical School, Nagasaki, Japan.*

Kunkel, Beverly Waugh, Ph.B., Ph.D., Professor of Biology, *Lafayette College, Easton, Pa.*

Kuntz, Albert, Ph.D., M.D., Professor of Anatomy and Biology, *St. Louis University Medical School, 1402 South Grand Ave., St. Louis, Mo.*

Kutchin, Mrs. Harriet Lehmann, A.M., *"The Maplewood," Green Lake, Wis.*

Lambert, Adrian V. S., A.B., M.D., Clinical Professor of Surgery, Columbia University, *168 East Seventy-first St., New York City.*

Lambert, Avery Eldorus, B.S., Ph.D., Professor of Anatomy, Department of Anatomy, University of Iowa, *Room 360, Medical Laboratories, Iowa City, Iowa.*

Landacre, Francis Leroy, Ph.D., Professor of Anatomy and Head of Department, Ohio State University, *2026 Inka Ave., Columbus, Ohio.*

Langworthy, Orthello R., A.B., A.M., M.D., Associate in Neurology, Johns Hopkins University, *Johns Hopkins Hospital, Baltimore, Md.*

Larsell, Olof, A.M., Ph.D., Professor of Anatomy, *University of Oregon Medical School, Portland, Oregon.*

Latimer, Homer B., A.M., Ph.D., Professor of Anatomy, *Medical School, University of Kansas, Lawrence, Kansas.*

Latta, John S., A.B., Ph.D., Professor of Anatomy, University of Nebraska, College of Medicine, *Forty-second St. and Dewey Ave., Omaha, Neb.*

Lee, Ferdinand Christian, A.B., M.D., Associate in Surgery, *Johns Hopkins Hospital, Baltimore, Md.*

Lee, Thomas G., B.S., M.D. (Ex. Com. '08–'10, Vice-Pres. '12–'14), Emeritus Professor of Comparative Anatomy, Institute of Anatomy, University of Minnesota, *Babson Park, Florida.*

Leidy, Joseph, Jr., A.B., A.M., M.D., *1713 Walnut St., Philadelphia, Pa.*

Levi, Giuseppe, M.D., Professor of Anatomy, *University of Torino, Istituto anatomico, Corso d'Azeglio 52, Torino, Italy.*

Lewis, Dean D., M.D., Professor of Surgery, Johns Hopkins University, *Johns Hopkins Hospital, Baltimore, Md.*

Lewis, Frederic T., A.M., M.D. (Ex. Com. '09–'13, Vice-Pres. '14–'16), Associate Professor of Embryology, *Harvard Medical School, Boston, Mass.*

Lewis, Margaret Reed, M.A., Collaborator, Department of Embryology, Carnegie Institution of Washington, *Johns Hopkins Medical School, Baltimore, Md.*

Lewis, Warren Harmon, B.S., M.D. (Ex. Com. '09–'11, '14–'17), Research Associate, Department of Embryology, Carnegie Institution, Professor of Physiological Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*

Lillie, Frank Rattray, Ph.D., Sc.D., Professor of Embryology, Chairman of Department of Zoölogy, University of Chicago; Director Marine Biological Laboratory, Woods Hole, Mass., *University of Chicago, Chicago, Ill.*

Lineback, Paul Eugene, A.B., M.D., Professor of Microscopic Anatomy, *Atlanta Medical College, Emory University, Ga.*

Linell, Eric Ambrose, Ch.B., M.D., Assistant Professor of Anatomy, *University of Toronto, Toronto, Canada.*

Lipschutz, Benjamin, M.D., Demonstrator in Neuro-anatomy, Jefferson Medical College, *1007 Spruce St., Philadelphia, Pa.*

Little, Clarence Cook, B.A., M.S., Sc.D., LL.D., Director of Laboratory of Mammal Genetics, *University of Michigan, Ann Arbor, Michigan.*

Looper, James Burdine, B.S., M.S., Ph.D., Associate Professor of Histology and Embryology, *Box 55, University of Mississippi, University, Miss.*

Lord, Frederic P., A.B., M.D., Professor of Anatomy, *Dartmouth Medical School, 39 College St., Hanover, N. H.*

Loth, Edward, M.D., Ph.D., Professor of Anatomy and Director of Anatomical Institute, *University of Warsaw, Warsaw, Poland.*

Lowrey, Lawson Gentry, A.M., M.D., Director, Institute for Child Guidance, National Committee for Mental Hygiene, *9th Floor, 145 East 57th Street, New York City.*

MacCallum, Daniel Bartlett, S.B., Ph.D., M.D., Instructor, *Department of Internal Medicine, University of Wisconsin, Madison, Wisconsin.*

MacFarland, Frank Mace, A.M., Ph.D., Professor of Histology, Leland Stanford Junior University, *775 Santa Ynez Street, Stanford University, Calif.*

McClendon, Jesse F., Ph.D., Professor of Physiological Chemistry, *University of Minnesota, Minneapolis, Minn.*

McClung, Clarence E., Ph.G., A.B., A.M., Ph.D., Professor of Zoölogy and Director of the Zoölogical Laboratory, *University of Pennsylvania, Philadelphia, Pa.*

McClure, Charles Freeman Williams, A.M., Sc.D. (Vice-Pres. '10–'11, Ex. Com. '12–'16, Pres. '20–'21), Professor of Comparative Anatomy, *Princeton University, Princeton, N. J.*

McCotter, Rollo E., M.D., Professor of Anatomy, Medical Department, University of Michigan, *1043 Ferdon Road, Ann Arbor, Mich.*

McGill, Caroline, A.B., A.M., Ph.D., M.D., Physician, *58 W. Quartz, Butte, Mont.*

McKibben, Paul S., Ph.D., F.R.S.C., Professor of Anatomy, *University of Southern California, Los Angeles, Calif.*

McMurrich, James Playfair, A.M., Ph.D., LL.D. (Ex. Com. '06–'07, '17–'20, Pres. '08–'09), Professor of Anatomy, *University of Toronto, Toronto, Canada.*

McNutt, G. W., D.V.M., Assistant Professor of Anatomy, *College of Veterinary Medicine, Washington State College, Pullman, Wash.*

Macklin, C. C., M.B., M.D., M.A., Ph.D., F.R.S.C. (Ex. Com. '28–), Professor and Chief of the Department of Anatomy, *University of Western Ontario Medical School, London, Ontario, Canada.*

Magath, Thomas Byrd, M.S., Ph.D., M.D., Associate in Clinical Laboratories, University of Minnesota, *Mayo Clinic, Rochester, Minn.*

Mainland, Donald, M.B., Ch.B., Assistant Professor of Anatomy, *Department of Anatomy, University of Manitoba, Winnipeg, Canada.*

Malone, Edward F., A.B., M.D., Francis Brunning Professor of Anatomy, *University of Cincinnati, College of Medicine, Eden Ave., Cincinnati, Ohio.*

Mangum, Charles S., A.B., M.D., Professor of Anatomy, *University of North Carolina, 409 East Franklin St., Chapel Hill, N. C.*

Mark, Edward Laurens, Ph.D., LL.D., Hersey Professor of Anatomy (Emeritus) and Director of the Bermuda Biological Station for Research, Harvard University, *109 Irving St., Cambridge, Mass.*

Markee, J. E., Ph.D., Instructor, *Department of Anatomy, Stanford University, Stanford, California.*

Mason, Karl E., Ph.D., Instructor in Anatomy, *Vanderbilt University Medical School, Nashville, Tenn.*

Mastin, Edward V., M.S., M.D., Instructor in Anatomy, *1043 Missouri Building, St. Louis, Mo.*

Matas, Rudolph, M.D., LL.D., Emeritus Professor of Surgery and Demonstrator of Anatomy, Tulane University of Louisiana, *2255 St. Charles Ave., New Orleans, La.*

Matthews, Samuel A., M.A., Ph.D., Instructor in Anatomy, *Medical Department, University of Pennsylvania, Philadelphia, Penna.*

Mattingly, Marie Dees, M.D., Assistant Professor of Anatomy, *Tulane University of Louisiana, New Orleans, La.*

Mavor, James Watt, B.A., M.A., Ph.D., Professor of Biology, *Union College, Schenectady, N. Y.*

Mead, Harold Tupper, B.A., M.S., Professor of Zoölogy, *Hartwick College, Oneonta, N. Y.*

Meaker, Samuel R., A.B., M.D., M.R.C.S. (Eng.), F.A.C.S., Professor of Gynaecology, Boston University School of Medicine, *475 Commonwealth Ave., Boston, Mass.*

Meyer, Adolf, M.D., LL.D., Professor of Psychiatry and Director of the Phipps Psychiatric Clinic, *Johns Hopkins Hospital, Baltimore, Md.*

Meyer, Arthur W., S.B., M.D. (Ex. Com. '12–'16, Vice-Pres. '24–'25), Professor of Anatomy, Leland Stanford Junior University, *121 Waverly St., Palo Alto, Calif.*

Michels, Nicholas A., M.A., D.Sc., Associate Professor of Anatomy, *The Daniel Baugh Institute of Anatomy, Jefferson Medical College, Philadelphia, Pa.*

Miller, Adam M., A.M., Dean and Professor of Anatomy, Long Island College Hospital, *335 Henry St., Brooklyn, N. Y.*

Miller, Shirley P., A.B., A.M., Ph.D., Assistant Professor of Anatomy, *Anatomical Institute, University of Minnesota, Minneapolis, Minn.*

Miller, William Snow, M.D., Sc.D. (Vice-Pres. '08–'09), Professor of Anatomy (Emeritus), University of Wisconsin, *2001 Jefferson St., Madison, Wis.*

Moody, Robert Orton, B.S., M.D., Associate Professor of Anatomy, University of California, *2826 Garber St., Berkeley, Calif.*

Moore, Carl R., A.M., Ph.D., Professor of Zoölogy, *Hull Zoölogical Laboratory, University of Chicago, Chicago, Ill.*

Morgan, Lawrence Onis, A.B., A.M., Ph.D., Assistant Professor of Anatomy, *University of Cincinnati, College of Medicine, Eden Ave., Cincinnati, Ohio.*

Morrill, Charles V., A.M., Ph.D., Associate Professor of Anatomy, *Cornell University Medical School, First Ave. and Twenty-eighth St., New York City.*

Morton, Dudley Joy, M.D., Associate Professor, Department of Anatomy, *College of Physicians and Surgeons, Columbia University, 630 West 168th St., New York City.*

Mossman, Harland W., Ph.D., Assistant Professor of Anatomy, *Science Hall, University of Wisconsin, Madison, Wis.*

Mull, Philip L., B.S., M.D., Professor of Anatomy, *University of Mississippi Medical School, Box 102, University, Miss.*

Murray, H. A., Jr., A.B., A.M., M.D., Ph.D., Assistant Professor of Psychology, *Harvard University, Cambridge, Mass.*

Murray, William S., B.A., M.A., Sc.D., Investigator, Roscoe J. Jackson Memorial Laboratory, *Box 148, Bar Harbor, Maine.*

Myers, Burton D., A.M., M.D., Professor of Anatomy and Dean of the Indiana University School of Medicine, *424 N. Walnut St., Bloomington, Ind.*

Myers, Jay A., M.S., Ph.D., M.D., Assistant Professor of Preventive Medicine, *University of Minnesota Medical School, Minneapolis, Minn.*

Myers, Mae Lichtenwalner, M.D., Professor of Histology and Embryology, *Women's Medical College of Pennsylvania, 2101 North College Ave., Philadelphia, Pa.*

Nachtrieb, Henry Francis, B.S., Professor Emeritus, Animal Biology, University of Minnesota, *2448 Cedar St., Berkeley, Calif.*

Nadgir, Y. G., M.D., Professor of Anatomy, *Grant Medical College, Bombay, India.*

Nañagas, Juan Cancio, M.D., Professor of Anatomy, College of Medicine, University of the Philippines, *719 Florida St., Manila, Philippine Islands.*

NEAL, HERBERT VINCENT, A.M., Ph.D., Professor of Zoölogy, Tufts College, *Tufts College, 57, Mass.*

NICHOLAS, JOHN SPANGLER, M.S., Ph.D., Assistant Professor of Anatomy, *Osborn Zoölogical Laboratory, Yale University, New Haven, Conn.*

NOBACK, GUSTAVE J., B.S., M.A., Ph.D., Associate Professor of Anatomy, New York University and Bellevue Medical College, *338 East Twenty-sixth St., New York City.*

NOBLE, GLADWIN K., A.B., M.A., Ph.D., Curator of Herpetology, *American Museum of Natural History, New York City.*

NONIDEZ, JOSÉ F., M.S., Sc.D., Assistant Professor of Anatomy, Cornell University Medical College, *477 First Ave., New York City.*

NORRIS, H. W., A.B., Professor of Zoölogy, *Grinnell College, Grinnell, Iowa.*

OBENCHAIN, JEANETTE B., Ph.D., Research Associate, Department of Anatomy, *University of Chicago, Chicago, Ill.*

O'DONOGHUE, CHARLES H., D.Sc., F.Z.S., Lecturer, *Department of Zoölogy, University of Edinburgh, Scotland.*

OKAJIMA, K., M.D., Professor of Anatomy, *Keio University Medical College, Yotsuya, Tokio, Japan.*

O'LEARY, JAMES L., Ph.D., Assistant Professor of Cytology, *Washington University School of Medicine, St. Louis, Mo.*

OSTERUD, HJALMAR L., A.B., A.M., Ph.D., Professor of Anatomy, *Medical College of Virginia, Richmond, Va.*

OVERHOLSER, MILTON D., A.B., A.M., Ph.D., Instructor, *Department of Anatomy, University and Bellevue Hospital Medical College, New York University, 338 East Twenty-sixth St., New York City.*

PAINTER, THEOPHILUS S., Ph.D., Adjunct Professor of Zoölogy, *School of Zoölogy, University of Texas, Austin, Texas.*

PALMER, CARROLL E., M.A., M.D., National Research Council Fellow, *Division of Child Welfare, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Md.*

PAPANICOLAOU, GEORGE N., Ph.D., M.D., Assistant Professor of Anatomy, *Cornell University Medical College, Twenty-eighth St. and First Ave., New York City.*

PAPEZ, JAMES WENCESLAS, B.A., M.D., Assistant Professor of Anatomy and Neurology, *Cornell University Medical College, Ithaca, N. Y.*

PARKER, GEORGE HOWARD, D.Sc., Professor of Zoölogy and Director of Zoölogical Laboratory, *Harvard University, 16 Berkeley St., Cambridge, Mass.*

PATON, STEWART, A.B., M.D., *The Warrington Apartments, 3908 N. Charles St., Baltimore, Md.*

PATTEN, BRADLEY MERRILL, A.M., Ph.D., Associate Professor of Histology and Embryology, School of Medicine, Western Reserve University, *2109 Adelbert Road, Cleveland, Ohio.*

PATTEN, WILLIAM, Ph.D., Professor of Zoölogy, *Dartmouth College, Hanover, N. H.*

PATTERSON, JOHN THOMAS, B.S., Ph.D., Professor and Chairman of the Department of Zoölogy, University of Texas, *1908 Cliff St., Austin, Texas.*

PERKINS, ORMAN C., A.B., A.M., M.D., Professor of Neurology, Long Island College Hospital, *829 Carroll St., Brooklyn, N. Y.*

PIERSOL, WILLIAM HUNTER, A.B., M.B., Professor of Histology and Embryology, University of Toronto, *35 Dunvegan Road, Toronto, Canada.*

Pohlman, Augustus G., M.D., Professor of Anatomy, St. Louis University, School of Medicine, *1402 South Grand Ave., St. Louis, Mo.*

Poljak, Stephen, M.D., Assistant Professor of Neuro-anatomy, *Department of Anatomy, University of California, Berkeley, Calif.*

Potter, Peter, M.S., M.D., Oculist and Aurist, Murray Hospital, Butte, Montana, *125 W. Granite St., Butte, Montana.*

Poynter, Charles W. M., B.S., M.D. (Ex. Com. '19–'22), Professor of Anatomy, College of Medicine, University of Nebraska, *Forty-second and Dewey Ave., Omaha, Neb.*

Prentiss, H. J., M.D., M.E., Professor of Anatomy, Embryology, and Histology, *State University of Iowa, Iowa City, Iowa.*

Pryor, Joseph William, M.D., Emeritus Professor of Anatomy and Physiology, University of Kentucky, *417 West Second St., Lexington, Ky.*

Radasch, Henry E., M.S., M.D., Professor of Histology and Embryology, Jefferson Medical College, *Daniel Baugh Institute of Anatomy, Eleventh and Clinton Sts., Philadelphia, Pa.*

Ranson, Stephen W., M.D., Ph.D. (Ex. Com. '21–'24, Vice-Pres. '26–'28), Director Neurological Institute, *Northwestern University Medical School, 303 East Chicago Ave., Chicago, Ill.*

Rasmussen, Andrew T., Ph.D., Professor of Neurology, *Institute of Anatomy, University of Minnesota, Minneapolis, Minn.*

Raven, Harry C., B.A., Associate Curator, Department of Comparative and Human Anatomy, *American Museum of Natural History, Seventy-seventh St. and Central Park West, New York City.*

Reagan, Franklin P., Ph.D., Associate Professor of Anatomy, *Indiana University Medical School, Bloomington, Ind.*

Reed, Hugh D., B.S., Ph.D., Professor of Zoölogy, Cornell University, *McGraw Hall, Ithaca, N. Y.*

Reinke, Edwin E., M.A., Ph.D., Professor of Biology, *Vanderbilt University, Nashville, Tenn.*

de Rényi, George St., M.D., Associate Professor of Anatomy, *School of Medicine, University of Pennsylvania, Philadelphia, Pa.*

Revell, Daniel Graisberry, A.B., M.B., Professor of Anatomy, *University of Alberta, Edmonton, Alberta, Canada.*

Rhinehart, D. A., A.M., M.B., Professor of Applied Anatomy, University of Arkansas, *1624 West Twenty-first St., Little Rock, Ark.*

Rice, Edward Loranus, A.B., Ph.D., Sc.D., Professor of Zoölogy, *Ohio Wesleyan University, Delaware, Ohio.*

Rienhoff, William F., Jr., A.B., M.D., Instructor in Surgery, *Johns Hopkins Hospital, Baltimore, Md.*

Ringoen, Adolph R., A.M., Ph.D., Associate Professor of Zoölogy, *Department of Zoölogy, University of Minnesota, Minneapolis, Minn.*

Robertson, Albert Duncan, B.A., M.A., Professor of Zoölogy, *Western University, London, Ontario, Canada.*

Robinson, Arthur, M.D., F.R.C.S. (Edinburgh), Professor of Anatomy, University of Edinburgh, *University, Teviot Place, Edinburgh, Scotland.*

Robinson, Byron L., B.A., M.B., M.A., M.D., Professor of Microscopic Anatomy, *University of Arkansas Medical School, Little Rock, Ark.*

ROGERS, WILLIAM MITCHELL, B.S., Instructor in Anatomy, *College of Physicians and Surgeons, Columbia University, 630 West 168th St., New York City.*

ROMER, ALFRED S., B.A., Ph.D., Associate Professor of Vertebrate Palaeontology, *Walker Museum, University of Chicago, Chicago, Ill.*

RUPERT, R. R., M.D., *532 Fifteenth St., Oakland, Calif.*

RUTH, EDWARD S., M.D., Associate Surgeon, Children's Hospital, *Suite 416–417 Dalziel Building, 532 Fifteenth St., Oakland, Calif.*

SABIN, FLORENCE R., B.S., M.D., Sc.D. (Second Vice-Pres. '08–'09, Pres. '24–'25), Member of Rockefeller Institute, *Rockefeller Institute for Medical Research, Sixty-sixth St. and Avenue A, New York City.*

SACHS, ERNEST, A.B., M.D., Professor of Clinical and Neurological Surgery, *Washington University School of Medicine, St. Louis, Mo.*

SANTEE, HARRIS E., A.M., Ph.D., M.D., Neurologist, *R. F. D., Box 72, West Chicago, Ill.*

SCAMMON, RICHARD E., Ph.D. (Ex. Com. '23–'26, Vice-Pres. '28–'30), Professor of Anatomy, *Institute of Anatomy, University of Minnesota, 33 Arthur Ave., S. E., Minneapolis, Minn.*

SCHAEFFER, JACOB PARSONS, A.M., M.D., Ph.D., Sc.D. (Ex. Com. '24–'29), Professor of Anatomy and Director of the Daniel Baugh Institute of Anatomy, Jefferson Medical College, *4634 Spruce St., Philadelphia, Pa.*

SCHNEIDER, ANTOINE J., M.D., Instructor in Anatomy, *University of Rochester School of Medicine, Rochester, N. Y.*

SCHOCHET, SYDNEY S., M.D., Surgeon, *55 East Washington St., Chicago, Ill.*

SCHOEMAKER, DANIEL M., B.S., M.D., Professor of Anatomy, Medical Department, St. Louis University, *1402 South Grand Ave., St. Louis, Mo.*

SCHULTE, HERMANN VON W., A.B., M.D. (Ex. Com. '15–'18), Professor of Anatomy and Dean, Creighton Medical College, *306 North Fourteenth St., Omaha, Neb.*

SCHULTZ, ADOLPH H., Ph.D., Associate Professor of Physical Anthropology, Department of Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*

SCOTT, GORDON H., Ph.D., Assistant Professor of Cytology, *Washington University School of Medicine, St. Louis, Mo.*

SCOTT, JOHN W., A.B., Ph.D., Professor of Zoölogy and Research Parasitologist, *University of Wyoming, Laramie, Wyoming.*

SELLING, LAWRENCE, A.B., M.D., Clinical Professor of Medicine, University of Oregon Medical School, *Mayer Building, Portland, Ore.*

SENIOR, H. D., M.D., D.Sc., F.R.C.S. (Vice-Pres. '22–23), Professor of Anatomy, New York University and Bellevue Hospital Medical College, *338 East Twenty-sixth St., New York City.*

SEVERINGHAUS, AURA E., B.S., M.A., Ph.D., Assistant Professor of Anatomy, *College of Physicians and Surgeons, Columbia University, New York City.*

SHANER, RALPH FAUST, Ph.D., Professor of Anatomy, *University of Alberta, Edmonton, Alberta, Canada.*

SHARP, CLAYTON, A.B., M.D., Assistant Professor of Anatomy, Hoagland Laboratory, Long Island College Hospital, *335 Henry St., Brooklyn, New York.*

SHELLSHEAR, JOSEPH LEXDEN, M.B., Ch.M., Professor of Anatomy, *University of Hongkong, Hongkong, China.*

SHIELDS, RANDOLPH TUCKER, A.B., M.D., Professor of Histology and Embryology, School of Medicine, *Shantung Christian University, Tsinan, Shantung, China.*

SHIMIDZU, YOSHITAKA, M.D., Professor of Gynecology, *Nagasaki Medical College, Nagasaki, Japan.*

SHUMWAY, WALDO, A.B., A.M., Ph.D., Professor of Zoölogy and Assistant Dean, College of Liberal Arts and Sciences, *University of Illinois, Urbana, Ill.*

SIMKINS, CLEVELAND S., A.B., Ph.D., Associate Professor of Anatomy, Histology, and Embryology, University of Tennessee Medical School, *875 Monroe Ave., Memphis, Tenn.*

SIMPSON, MIRIAM ELIZABETH, A.B., M.S., Ph.D., M.D., Instructor in Anatomy, *University of California, Berkeley, Calif.*

SMITH, BERTRAM GARNER, A.B., Ph.D., Associate Professor of Anatomy, New York University Medical College, *338 East Twenty-sixth St., New York City.*

SMITH, CHRISTIANNA, A.M., Associate Professor of Zoölogy, *Mount Holyoke College, South Hadley, Mass.*

SMITH, DAVID T., A.B., M.D., Bacteriologist, New York State Hospital for Incipient Tuberculosis, *Ray Brook, N. Y.*

SMITH, GEORGE MILTON, A.B., M.D., Attending Surgeon, St. Mary's and Waterbury Hospitals, *111 Buckingham St., Waterbury, Conn.*

SMITH, GRAFTON ELLIOT, M.A., M.D., F.R.S., Ch.M., L.D., F.R.C.P., Professor of Anatomy, *University College, Gower Street, London, W.C. 1, England.*

SMITH, H. P., A.B., M.S., M.D., Assistant Professor of Pathology, *University of Rochester School of Medicine and Dentistry, Elmwood Ave., Rochester, N. Y.*

SMITH, PHILIP EDWARD, M.S., Ph.D., Professor of Anatomy, *Columbia University Medical School, 632 West 168th St., New York City.*

SMITH, WILBUR CLELAND, M.D., Professor of Gross Anatomy, *Tulane University, New Orleans, La.*

SNOW, PERRY G., A.B., M.D., Professor of Anatomy, *University of Utah Medical School, Salt Lake City, Utah.*

SNYDER, FRANKLIN FAUST, M.D., Assistant, Department of Obstetrics, *Johns Hopkins Hospital, Baltimore, Md.*

SPAULDING, M. H., A.B., A.M., Professor of Zoölogy, University of Montana, *507 South Eighth Ave., Bozeman, Montana.*

SPEIDEL, CARL C., Ph.B., Ph.D., Associate Professor of Anatomy, University of Virginia, *University, Va.*

STALEY, FOREST H., A.B., M.D., Instructor in Surgery, *St. Louis University School of Medicine, St. Louis, Mo.*

STARK, MARY, Ph.B., M.A., Ph.D., Professor of Histology and Embryology, *New York Homeopathic College, Sixty-fourth St. and Avenue A, New York City.*

STEVENSON, PAUL H., B.S., M.D., Associate in Anatomy, *Peking Union Medical College, Peking, China.*

STEWART, FRED WALDORF, A.B., Ph.D., M.D., Associate, Division of Pathology and Bacteriology, *The Rockefeller Institute for Medical Research, New York City.*

STILES, HENRY WILSON, M.D., Professor of Anatomy, College of Medicine, Syracuse University, *309 Orange St., Syracuse, N. Y.*

STOCKARD, CHARLES RUPERT, M.D., M.S., Ph.D., Sc.D. (Secretary-Treasurer '14–'21, Ex. Com. '23–'28, Pres. '28–'30), Professor of Anatomy, *Cornell University Medical College, New York City.*

STONE, LEON STANSFIELD, Ph.B., Ph.D., Associate Professor of Anatomy, Yale School of Medicine, *Sterling Hall of Medicine, Anatomy Department, Cedar St., New Haven, Conn.*

STONE, ROBERT S., B.A., M.A., M.B., M.D., Assistant Professor of Roentgenology, University of California, University Hospital, *Parnassus and Third Aves., San Francisco, Calif.*

STOPFORD, JOHN SEBASTIAN B., M.D., Professor of Anatomy, *University of Manchester, Manchester, England.*

STRAUS, WILLIAM L., JR., Ph.D., Instructor in Anatomy, Johns Hopkins University, *Johns Hopkins Medical School, Baltimore, Md.*

STREETER, GEORGE L., A.M., M.D., Sc.D. (Ex. Com. '18–'20, Pres. '26–'28), Director, Department of Embryology, Carnegie Institution of Washington, *Johns Hopkins Medical School, Baltimore, Md.*

STROMSTEN, FRANK ALBERT, D.Sc., Associate Professor of Animal Biology, State University of Iowa, *318 Zoölogy Building, Iowa City, Iowa.*

STRONG, LEON H., Ph.D., Assistant Professor of Anatomy, Medical School, University of Michigan, *1008 E. Medical Building, University of Michigan, Ann Arbor, Mich.*

STRONG, OLIVER S., A.B., A.M., Ph.D., Professor of Neurology and Neuro-histology, Columbia University, *College of Physicians and Surgeons, 630 West 168th St., New York City.*

STRONG, REUBEN MYRON, A.B., A.M., Ph.D. (Ex. Com. '16–'19), Professor of Anatomy, *Loyola University School of Medicine, 706 South Lincoln St., Chicago, Ill.*

STUNKARD, H. W., Ph.D., Professor of Biology, *New York University, New York City.*

SULLIVAN, WALTER EDWARD, A.M., Ph.D., Professor of Anatomy, University of Wisconsin, *Science Hall, Madison, Wis.*

SUNDWALL, JOHN, Ph.D., M.D., Professor of Hygiene, Director of Hygiene, Public Health and Physical Education, University of Michigan, *619 East University Ave., Ann Arbor, Mich.*

SUTTON, ALAN CALLENDER, A.B., M.D., Physician, *5600 Pimlico Road, Baltimore, Md.*

SWENSON, E. A., A.B., A.M., Ph.D., Instructor in Anatomy, *Medical Department, University of Pennsylvania, Philadelphia, Penna.*

SWETT, FRANCIS HUNTINGTON, A.M., Ph.D., Associate Professor of Anatomy, *Vanderbilt Medical School, Nashville, Tenn.*

SWIFT, CHARLES H., M.D., Ph.D., Assistant Professor of Anatomy, *Department of Anatomy, University of Chicago, Chicago, Ill.*

SWINGLE, W. W., A.B., A.M., Ph.D., Professor of Biology, *Princeton University, Princeton, N. J.*

SYKES, GEO. F., Ph.B., M.A., Assistant Professor of Anatomy, *Tufts College Medical School, Boston, Mass.*

TERRY, ROBERT JAMES, A.B., M.D. (Ex. Com. '08–'12, '21–'24), Professor of Anatomy, *Washington University Medical School, St. Louis, Mo.*

THOMPSON, IAN MACLAREN, B.Sc., M.B., Ch.B., Associate Professor of Anatomy, *University of California, Berkeley, Calif.*

THOMSON, ARTHUR, M.A., M.B., LL.D., F.R.C.S., D.C.L., Professor of Anatomy, *University of Oxford, Department of Human Anatomy, Oxford, England.*

THORKELSON, JACOB, M.D., *20 West Granite St., Butte, Mont.*

THRO, WILLIAM C., B.S., A.M., M.D., Professor of Clinical Pathology, *Cornell University Medical College, Twenty-eighth St. and First Ave., New York City.*

THURINGER, JOSEPH M., M.D., Professor of Histology and Embryology, *University of Oklahoma, School of Medicine, Oklahoma City, Okla.*

THYNG, FREDERICK WILBUR, Ph.D., Professor of Anatomy, *Tufts Medical College, 416 Huntington Ave., Boston, Mass.*

TILNEY, FREDERICK, A.B., M.D., Ph.D., Professor of Neuro-Anatomy, Columbia University, *870 Madison Ave., New York City.*

TODD, T. WINGATE, M.B., Ch.B. (Manc.), F.R.C.S. (Eng.), (Vice-Pres. '20–'21, Ex. Com. '24–'29), Professor of Anatomy, Director of the Hamann Museum of Comparative Anthropology and Anatomy, Medical Department, *Western Reserve University, 2109 Adelbert Road, Cleveland, Ohio.*

TOWER, SARAH SHELDON, B.S., Ph.D., M.D., Instructor in Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*

TRACY, HENRY C., A.B., A.M., Ph.D., Professor of Anatomy, *University of Kansas, Lawrence, Kansas.*

TROTTER, MILDRED, A.B., M.S., Ph.D., Assistant Professor in Anatomy, *Washington University Medical College, St. Louis, Mo.*

TURNER, CLARENCE L., M.A., Ph.D., Associate Professor of Zoölogy, *Northwestern University, Evanston, Ill.*

UHLENHUTH, EDUARD, Ph.D., Associate Professor in Anatomy, *University of Maryland Medical School, Baltimore, Md.*

VAN CAMPENHOUT, ERNEST, M.D., Instructor in Anatomy, *Yale University School of Medicine, New Haven, Conn.*

VAN DER HORST, C. J., Ph.D., Senior Lecturer in Zoölogy, *Department of Zoölogy, University of Witwatersrand, Johannesburg, South Africa.*

VAN WAGENEN, GERTRUDE, A.B., Ph.D., *Department of Anatomy, Johns Hopkins Medical School, Baltimore, Md.*

WAITE, FREDERICK CLAYTON, A.M., Ph.D., Professor of Histology and Embryology, Western Reserve University School of Medicine, *2109 Adelbert Road, Cleveland, Ohio.*

WALLIN, IVAN E., M.A., D.Sc., Professor of Anatomy and head of department, *University of Colorado, College of Medicine, Denver, Colo.*

WALMSLEY, THOMAS, M.D., F.R.S.E., Professor of Anatomy, *Queens University of Belfast, Belfast, Ireland.*

WATERSTON, DAVID, M.A., M.D., F.R.C.S., Ed., Butte Professor of Anatomy, *University of St. Andrews, St. Andrews, Fife, Scotland.*

WATKINS, RICHARD WATKIN, B.S., M.D., *5608 Kenwood Ave., Chicago, Ill.*

WATT, JAMES CRAWFORD, M.A., M.D., Associate Professor of Anatomy, University of Toronto, *20 Hawthorne Ave., Toronto, Canada.*

WEATHERFORD, HAROLD LORRAINE, A.B., A.M., Ph.D., Instructor in Anatomy, *Harvard Medical School, Boston, Mass.*

WEED, LEWIS HILL, A.B., A.M., M.D., Sc.D. (Ex. Com. '20–'21, Sec.-Treas. '22–'30), Dean and Professor of Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*

WHEELER, THEODORA, A.B., M.D., *604 Fifth St., S. W., Rochester, Minn.*

WHITESIDE-HARVEL, BEATRICE, B.A., Ph.D., Voluntary Research Worker, *Anatomisch-biologisches Institut der Universität, Berlin, Germany.*

WHITNALL, S. E., M.A., M.D., B.Ch., Robert Reford Professor of Anatomy, *McGill University, Montreal, Canada.*

WIEMAN, H. L., Ph.D., Professor of Zoölogy, *University of Cincinnati, Cincinnati, Ohio.*

WILLARD, WILLIAM A., A.M., Ph.D., Professor of Anatomy, University of Nebraska, *College of Medicine, Forty-second St. and Dewey Ave., Omaha, Neb.*

WILLIER, BENJAMIN HARRISON, Ph.D., Associate Professor of Zoölogy, *University of Chicago, Chicago, Ill.*

WILSON, JAMES THOMAS, M.A., M.B., F.R.S., Professor of Anatomy, *University of Cambridge, New Museums, Cambridge, England.*

WILSON, LOUIS BLANCHARD, M.D., Director of Pathology Division, Mayo Clinic and Mayo Foundation, Professor of Pathology in the University of Minnesota, *Mayo Clinic, 830 West College St., Rochester, Minn.*

WINDLE, WILLIAM F., B.S., M.S., Associate Professor of Anatomy, *Northwestern University Medical School, 303 East Chicago Ave., Chicago, Ill.*

WINKENWERDER, WALTER L., S.B., M.D., Instructor in Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*

WISLOCKI, GEORGE B., A.B., M.D., Associate Professor of Anatomy, *Johns Hopkins Medical School, Baltimore, Md.*

WITHERSPOON, THOMAS CASEY, M.D., *Murray Hospital, Butte, Mont.*

WITSCHI, EMIL, Ph.D., Professor of Zoölogy, *The State University of Iowa, Iowa City, Iowa.*

WITTENBORG, A. H., M.D., Professor of Anatomy, *College of Medicine, University of Tennessee, Memphis, Tenn.*

WOODMAN, ALICE S., M.D., Assistant Professor of Histology and Embryology, *Boston University School of Medicine, 80 East Concord St., Boston, Mass.*

WOOLLARD, HERBERT H., M.D., Professor of Anatomy, *St. Bartholomew's Hospital Medical School, London, England.*

WORCESTER, JOHN LOCKE, M.D., Professor of Anatomy, University of Washington, *5211 Twenty-first Ave., N. E., Seattle, Wash.*

ZIMMERMAN, ARNOLD, Ph.D., D.Sc., Assistant Professor of Anatomy, *Department of Anatomy, Loyola University School of Medicine, 706 South Lincoln St., Chicago, Ill.*

ZWEMER, RAYMOND L., A.B., Ph.D., Instructor in Anatomy, *College of Physicians and Surgeons, Columbia University, New York City.*

AN ATTEMPT TO IMPROVE THE METHODS OF ANATOMICAL TEACHING, INCLUDING THE ORGANIZATION OF THE DISSECTION TO AN UNUSUAL DEGREE BY SYSTEMS AND THE BRINGING OF THE DEVELOPMENTAL, GROSS, AND MICROSCOPIC ANATOMY OF INDIVIDUAL ORGANS TOGETHER IN THE SCHEDULE

E. D. CONGDON
Faculty of Medicine, Chulalankarana University, Bangkok, Siam

A favorable opportunity for attempting an improvement in the organization of anatomical instruction presented itself when the medical faculty of Chulalankarana University at Bangkok was raised to the status of a modern school by the Siamese government with the cooperation of the Rockefeller Foundation. There were made available good housing and an adequate supply of dissecting material, equipment, technical help, and teachers. The artistic talent of our Siamese assistants and technicians, their powers of patient application, and the delicacy and flexibility of their hands rendered them unusually efficient in the preparation of charts, dissections, and models.

The changes which were introduced into the teaching of anatomy have been directed toward two chief ends. The first is a determination of the order of dissection most suitable for rendering the facts of gross anatomy clear and easily comprehended, and the second, which is dependent upon the first for its accomplishment, is the bringing together of the gross, microscopic, and developmental anatomy of each organ to form a united picture in the student's mind. The resulting scheme may seem a disrespectful treatment of the traditional divisions of anatomy. Three years of experience with it, however, have left no doubt in the writer's mind that, if due allowance be made for certain local limitations in its application, the view which it has afforded the students of the facts and principles of anatomy is clearer and better proportioned than any in his previous teaching experience. The results of efforts to encourage self-activity tended to show that this goal can also be more largely attained in conjunction with a rigid synthetic course than when there is great freedom of arrangement of the dissecting program.

The internal organization of gross anatomy at Bangkok has been so planned that the sequence of structures dissected shall, as far as possible, parallel the order of scientific exposition. The scientific order of exposition of gross anatomy is by organ systems. If anatomical structures are thus grouped, they are easier to comprehend and remember, because they appear as adaptations to the functions of these systems. Although the intermingling of systems in the body is so great as to give topography also an importance in medical teaching, yet its facts have not such easily comprehendable functional explanations as those of systemic anatomy and are therefore on the whole less suitable for elementary study. The topographic must, therefore, to a certain extent give place to the systematic as gross anatomy is curtailed to accommodate it to the time limitations of an anatomical course.

If a student, after reading the description of a system in the text, could carry out its dissection in the order read, he would be spared the expenditure of a considerable part of the energy at present required in applying his reading to the laboratory work. But this would mean a dissection, system by system, and therefore an increase in the work of dissection which would more than offset the economy in relating his reading to the laboratory work. Also, topography would not receive as much emphasis as it deserves. It is desirable to find some arrangement which will bring the dissection into as close a parallel with the systematic presentation of the text-book as possible without a sacrifice of the student's energy or an undue consumption of dissecting material and without making great inroads on the study of topography.

Two familiar plans of dissection, the one exemplified in Germany and the other in England, differ greatly in the emphasis placed upon arrangement by systems and to a much lesser degree in the attention accorded by them to topography. The German scheme groups together three functionally related systems, the muscles, bones, and joints, by dissecting these in the body wall and extremities in a first course, while omitting the viscera. The total viscera of the body, including nerves, blood vessels, and lymphatics, are dissected on other material in a second course. The method approaches a strict dissection by systems to the extent of grouping together three functionally related systems, the muscular, osseous and articular, while the visceral systems are taken up independently. This is doubtless the closest approximation to a systematic dissection of body wall and extremities practicable under the limitations of time and material that go with anatomy teaching. Yet this method places great demands on a student's energy and upon the supply of anatomical material, since the body wall and extremities are twice dissected. In the English plan there is an economy in these respects, as only one body is used for the dissection. On the other hand, there is a thorough intermingling of visceral systems with muscles, bones, and joints, because head and neck, thorax, or abdomen may be assigned as dissection units or, in the case of two of these, may be combined with the corresponding extremities. Topography is given nearly equal attention in the two methods if one except the relation of body wall and surface to subjacent viscera. In this important topic the English method is superior, for in it alone is it the regular practice for the dissector to proceed to the study of the viscera under an area of body wall immediately after they have been exposed.

At Bangkok a compromise between the two methods is in use. The dissection of each group of students is confined to a single body, thus economizing both energy and dissecting material. At the same time dissection is carried out by organ systems to a degree that on the whole seems greater than in the German plan. The body wall and extremities are first studied in their entirety. This necessitates a mingling of nerves, blood vessels, and lymphatics with muscles, bones, and joints, and thus has less of segregation into systems as far as body and extremities are concerned than is to be found in the German method. Nevertheless, muscles, bones, and joints require such a preponderating amount of attention in the dissection of these parts that a high degree of unity in their study is made possible.

After the body wall and extremities have been completed, the deep-lying visceral systems of head, neck, and trunk, with the exception of the major part of the nervous system, receive attention. In arranging this part of the work the requirement was especially held in view that each system be dissected as far as possible as a unit unmixed with other systems. Because dissection of the viscera is not intermingled with the body wall, except to a slight degree in face and pelvis, it is possible to bring the order of their treatment into a rather close parallel with the systematic arrangement in the text-book. It has been found practicable to study several viscera of one system before the exigencies of dissection compel a turning to another system. Examples of parts of systems thus dissected together are: mouth cavity, oro- and laryngopharynx; thyroid, parathyroid, and thymus; heart, and large thoracic blood vessels, except descending aorta; larynx, trachea, and lungs. As an illustration of the degree of continuity attained in the study, it may be said that the entire digestive tract from oral cavity to rectum is completed with but three interruptions. These pauses between parts of systems are not of very long duration, because the total mass of body viscera is taken up continuously without any interjection of body wall and extremities.

Coherence in the treatment of visceral systems is further favored by the ordering of the organs of most systems in their logical functional sequence. Thus in the digestive system the oral cavity receives first attention, and the other parts, except for a slight displacement of pancreas, duodenum, and liver in the schedule, follow in functional order to the rectum. All of the respiratory system, except nose and nasopharynx, which are grouped with organs of special sense, are studied in the order: oral cavity, pharynx, larynx, trachea, and lungs. It can be seen that a very considerable degree of unity in the treatment of the visceral systems is attained by this plan and that their study must acquire an unusual momentum.

The completion of body wall before the viscera have been studied does not entail any great disturbance of the relation of the viscera to each other or to the body wall. They are still held in place by a shell of body wall, which permits an approximate understanding of their relation to overlying structures and to the surface. The deep dorsal wall has been left entirely undisturbed, because, for reasons which will be later explained, this region is taught from dissections made ready in advance by the staff. A considerable part of the lateral wall also remains after its dissection has been completed and even the sternum and linea alba can be replaced in nearly their original position, because care has been taken to leave the seventh costal cartilages intact. In spite of these considerations, a certain lack of emphasis upon the relation of body wall and subjacent viscera must be admitted. This is made good in the cross-section study of the second-year class. On the other hand, our arrangement adds one unusual advantageous feature to the topographic course. The topography of the larger viscera of the trunk exposed by the first-year class in their dissection is recorded by the second-year students in connection with their study of cross-sections. As these records are explained to the first-year class, each student gains familiarity with this feature of the cadavers of two successive years.

In the third and final division of the general course, termed 'neuro-anatomy,' an unusually large part of the nervous system finds united treatment, although most of the peripheral nerves have of necessity been already dissected in a scattering manner. The parts of the nervous system reserved till this time are: brain, spinal cord, eye, ear, nose, and deep dissection of the cranial nerves. The small part of the deep dissection of the head related to these structures and remaining after the study of the viscera must be completed at this time, although not a part of the nervous system.

Another opportunity to improve the organization of the dissection of body wall and extremities presents itself with reference to the progression from the easy to the difficult. This principle is, to be sure, recognized in most anatomy departments in the United States to the extent that more students begin with an upper extremity than with head and trunk. Yet even the upper extremity is far from offering the degree of simplicity desirable for the beginner. In Bangkok at the first of the dissection, when it is necessary to give many explanatory talks, the student is occupied with the removal of the upper extremity. There is thus a small amount of complex anatomy unavoidably placed at the very first of the course. The upper extremity is laid aside, however, as soon as removed from the body, and the course may be said to make its real beginning with the next dissection, which is of the anterior thoracic wall. It is thought desirable to take up the dissection of the body wall as soon as possible, because its metameric organization is readily comprehended by the beginner, who has just come from a course in mammalian anatomy. The thoracic wall is followed by the anterior abdominal wall and neck walls, thus passing to successively greater modifications of the primitive metameric structure. The anterior body wall of face and pelvis should come at this time also, were it not that they are in so close topographic and functional relation to deep-lying visceral systems that it is better to associate their study with these. The deep dorsal wall from sacrum to occiput is now taken up without interruption. As this completes the body wall except the two terminal parts just mentioned, the extremities are next dissected. Their position in the schedule with reference both to the body wall and to each other favors an effective comparison of their morphology.

The arrangement of the dissection which has been described renders anatomy easier to comprehend, chiefly because it is a functional order. The relation of function to the teaching of structure therefore may be considered. Leo Loeb long ago pointed out that physiology departments lack the material necessary for teaching much detailed function which would be of great value to the medical student and that the dissections required for this purpose lie ready at hand in the anatomical laboratory.

This claim of physiology for time allotted to anatomy might well be regarded askance by the anatomist in consideration of the great demands upon him of his own immediate subject, had it not long been clear that structure must be rationalized in order to fix it in the memory. This can be done most effectively by presenting it as an illustration of adaptation to function or by pointing out its clinical significance. Of the two means, function has the stronger claim even in a medical school, as a thorough understanding of the normal should precede consideration of the diseased. To be sure, the clinical has a dramatic appeal, but so also has functional anatomy if related to every-day life, to the range of variation of structure with age, sex, occupation, and race, and to interesting auxiliary material from various subjects. The very admirable project of bringing a certain amount of formal anatomical review into relation with the clinical work of the latter years of the medical course, as exemplified, for example, by the 'unit' system of teaching in England, harmonizes with a greater relinquishment of the clinical in first-year anatomy.

The emphasis given to dissection by systems at Bangkok and to arrangement of organs within each system in a functional order renders the use of function in teaching especially easy. Also, other features of the plan, as, for example, studying the body wall as a unit and grouping the extremities together, are favorable to consideration of correlated motor activities such as those of posture and respiration.

Because of previous familiarity with the English practice, it has been an interesting teaching experience for the writer to watch for three years the effects upon the students of emphasis upon systems and upon progression from the easy to the difficult—in which respects the English method is lacking. It has become very evident that these two changes of method afford a clearness in the presentation of the facts and principles of anatomy and its related special physiology not closely approached by the other plan.

The remaining changes in the methods of anatomy teaching have to do with the correlation of gross anatomy, microscopic anatomy, and embryology. These subjects are in most schools taught too independently of one another, and this is partly because of their recent rapid expansion. The form of correlation which has been effected at Bangkok is the bringing of the gross, microscopic, and developmental study of an organ near together or into immediate sequence in the schedule. The result is an unusual degree of blending of the total anatomy of an organ into a unit in the student's mind. Anatomy as a whole is thus made clearer and more interesting. We shall refer hereafter to this arrangement, for the sake of brevity, as the synthetic method.

With the exception of the nervous system, the study of the gross, microscopic, and developmental phases of an organ's structure forms part of the schedule of the first year in most medical schools of the United States. This association in time constitutes a certain degree of correlation of their respective contributions to its anatomy. Yet topics are presented in an order irregular and confusing from the viewpoint of synthesis by organs. In courses where the anatomy, exclusive of neurology, is spread over more than one year, the inadequacy of the correlation becomes yet more marked. The placing of neurology after the rest of the anatomy course is not, however, a great sin against correlation, as this subject has long been singled out for an approximately synthetic treatment.

The value of synthesis in the sense here used has doubtless suggested itself to nearly every teacher of anatomy at one time or another. The chief reason why the ideal has been so little realized is that the unsystematic arrangement of the dissection has offered an unsurmountable barrier. The laboratory work in embryology and in microscopic anatomy easily lends itself to arrangement by organ systems. If this be true also for the dissection, then little more is necessary than that the three subjects be run in parallel to group their contributions to the anatomy of an organ at one point in the schedule. The arrangement of the dissection by systems at Bangkok renders a thoroughgoing synthesis practicable. What elsewhere would be given as continuous courses in microscopic anatomy and embryology are here broken up into segments, each of which covers an organ or group of related organs dissected together, and the segments of the embryology and of microscopic anatomy are placed next to the corresponding dissection in the schedule. The study of embryology is, however, initiated by a continuous introductory part which includes the laying down of the organ systems, and microscopic anatomy begins with an uninterrupted study of cells and tissues.

The principle of synthesis cannot be applied with equal effectiveness to all parts of the body. It is very useful in its application to the deep-lying viscera of head, neck, and trunk, which, it will be remembered, are dissected in groups each representing a part of a system. It also works well in neuroanatomy, where organs of special sense are with one exception each treated as a unit, while the nervous system proper is divided only into central and peripheral parts. The method has less importance in the study of body wall and extremities, for here, because of the presence of many examples of each of the three organ types, muscle, bone, and joint, it is sufficient to associate together the developmental and microscopical anatomy of each type after a few examples have been dissected. And, finally, with reference to the numerous nerves, blood vessels, lymphatics, and fascial structures scattered throughout the body, it is obvious that synthesis is impossible except in the case of one important group of each. Thus for the haemovascular system, the dissection of the heart and large thoracic vessels is chosen for association with developmental and microscopical anatomy.

The sequence in which the three structural aspects of an organ should usually be arranged requires little debate and is indicated in a rough way by their relative position in most medical schedules. Development should usually come first, as its chief functions in the medical school, aside from its use as a preparation for obstetrics, are to aid in understanding the fundamental structural features of adult organs and to assist in explaining their variations. Gross anatomy should follow the embryology and microscopic anatomy come last. An exception to this order is occasionally necessary if the student has not a preliminary elementary knowledge of an organ group sufficient to insure an appreciation of its developmental history. In such cases a part of the dissection must precede the embryology.

The effect obtained by combining the proper order of presentation of the three phases of an organ structure with their immediate sequence or close association in the schedule will readily be appreciated by the teacher of anatomy. The developmental treatment is a helpful preparation for the dissection of an organ, and embryology and gross anatomy are an ideal preliminary for its microscopic anatomy. Even the embryology, although it usually comes first in order of the three subjects, is lent an added interest, because the organs studied have recently been exposed in the dissection and frequently related organs have already been dissected. The association of the three divisions of anatomy makes it possible to eliminate all duplication of topics treated by them. It automatically turns the attention of the instructors in the three subjects toward harmonizing and blending their presentations of the anatomy of each organ. Work on one subject suggests gaps to be filled in the others. For example, the combined study of mucous, serous, and synovial membranes in microscopic anatomy has called attention to the need of a preliminary comparison of them in the gross dissection.

The writer for a time feared that the lack of an even tempo in the schedules of microscopic anatomy and embryology necessitated by the correlation might cause the student to lose his perspective of the subjects as unit subsciences. More than the usual attention has therefore been given to independent reviews in both subjects. This drill and the introductory parts of microscopic anatomy and embryology taken together have been found to insure all of the independence in the view of these subjects that seems desirable.

As especial arrangement of the dissection outline is required in synthetic teaching. A rather brief laboratory and reading guide is used at Bangkok as an encouragement to self-activity, because it is felt that a manual, such as Cunningham's, by directing almost every taking up of the scalpel exerts a harmful influence in this respect. As the guide which was prepared lacks the great bulk of descriptive material found in some manuals, the student turns more to text, atlas, lectures, and scheduled laboratory talks. An unusual feature of the outline is a very complete page reference to topics in the text and to figures in text and atlas. It is believed that the time usually consumed by the student in looking up these matters affords him little return as a training in the use of literature and can be better employed. Every student is required to use an atlas for home study, as reading anatomy in the absence of anatomical preparations is at best a makeshift, and the situation should be alleviated by a ready access at home to a sufficient series of good anatomical illustrations to cover every structure whose description is read. The list of illustrations in the guide approaches closely a complete fulfillment of this requirement.

It may seem surprising to one who is impressed by the apparent informality of the synthetic scheme and its seeming lack of reverence for the classical divisions of anatomy that we have found it necessary to plan the schedule in advance to the extent that the daily work for the entire course has already been assigned its place at the beginning of the year. In this way each subdivision of anatomy may be readily made to present its contribution to the structure of an organ at the proper time. It is also believed that entirely aside from its usefulness in synthetic teaching, a fixed schedule is also necessary in order that the student may gain the well-proportioned survey of the facts and principles of anatomy which is rightly to be expected of him. There are several reasons why a fixed schedule is necessary for this purpose. It makes possible the thoughtful selection in advance of material from physiology, medicine, comparative anatomy, physical anthropology, and other sources which is most appropriate as an aid to each day's laboratory work. It is necessary if hours of drill are to be placed in strategical positions. It is of yet greater value in lessening the effort required to assign to matured laboratory talks, lectures, and demonstrations the exact locations in the schedule, day by day, where they will be of most help to the student. The definite scheduling of these aides far in advance is equally needed so that conditions will be most favorable for their improvement year by year. Anatomical science develops at such a pace that one need not fear that this method will result in unprogressive talks and lectures. Otherwise, they cannot attain their greatest excellence. It is believed that an efficiency engineer would look upon a fixed schedule as a necessity in carrying out the complicated requirements of a rich general course in anatomy.

There will always be difficulties in arriving at a teaching plan that emphasizes both the acquisition of a well-proportioned view of facts and principles of anatomy and the cultivation of habits of self-activity. A rather delicate adjustment of methods is required. When, as a reaction against didactic teaching, certain anatomists had the courage to leave a free rein to students in the dissecting room with little formal guidance, it was not only an arresting gesture, but it marked a real advance in the fostering of self-activity. Examples of this type of instruction are well known in the United States. In this change, however, the pendulum swung much too far. It would seem that the time had come for addressing oneself to the task of realizing the advantages of a fixed course as just recounted and at the same time evoking a maximum of self-activity.

Fortunately, a fixed course need not throttle a student's independence. Indeed, it is our belief that it is a necessary prerequisite for effective independent work. This is because it furnishes a much-needed preliminary grounding in the elements of the subject. The time of the anatomical course is too short and the accomplishment expected of a medical student too great to permit his independent thinking to be a rediscovery of the very rudiments of anatomy. It was found that the clear grasp of the basic facts of his dissection as assured by the fixed course makes it possible for the capable student to go further and independently recognize interesting features and problems of a less obvious nature. Stimulated by this recognition, if encouraged by the instructor, independent excursions will be taken by the more able students which may be so frequent as to give a fixed course a continuous flavor of independent observation and thought which will be far from 'lockstep' in character. One simple device found useful in this connection is a daily dissecting-room blackboard bulletin of interesting observations and thoughtful comments and questions by the students. A year's collection of these bulletins was found helpful in making plans for encouraging independence in succeeding years. A by-product of the encouragement of self-activity this year was the pointing out of nearly fifty errors in text and atlas by a class of twenty-four student. More important, however, than any mechanisms for stimulating self-activity is, of course, the mentality and personality of the teacher.

Consideration was given to the feasibility of abridging the fixed course in order to furnish time for additional work in which independence and individuality are especially emphasized. It was thought probable that the fixed course, even if somewhat shortened, would assure a sufficiently comprehensive acquaintance with the foundations of anatomy to justify such additional work. It was not found possible, however, to give this interesting scheme a trial within the time available for organizing the instruction at Bangkok before the publication of this paper.

A method by which the fixed course may be abbreviated to a certain degree has been in use for three years at Bangkok, although it was not introduced with the purpose of shortening the course as a whole. It was resorted to as a means of bringing the energy expenditure in the dissection of various parts of the body into closer harmony with their relative values to the student. It consists merely in setting aside the canon that all parts of the body must be dissected by each student, and teaching the parts omitted from the dissection with the help of preparations previously made ready by the technicians. Two considerations guide the selection of regions to be treated in this manner: first, that they require an especially great expenditure of energy for their dissection, and, secondly, that familiarity with them has less than average value to the student. The regions thus chosen are the subcutaneous planes of upper and lower extremities, deep dissection of dorsal body wall, total gluteal region and thigh, and the larynx. The time allowed for these dissections was reduced to 60 per cent of the usual allotment.

To the surprise of the instructors, the use of prepared dissections proved not to decrease the students' understanding of these parts. There have been available a sufficient number of good dissections so that the student has his usual amount of first-hand contact with them. The occasional shifts from cadaver to prepared parts rests the student by the change in the character of his work as well as by the cessation of the physical effort of dissection. Also, the use of prepared dissections introduces another marked deviation from the usual routine in that any part of a system represented can be studied in the text-book order without the usual interruptions necessitated by the removal of structures covering its deep-lying parts. As opportunities for day-dreaming while engaged in the mechanical work of dissection are absent and the time allowed for completing these regions is less than customary, more than the usual degree of concentration is required of the student. The writer does not argue for any considerable increase in the number of regions taught from prepared dissections over that so treated at Bangkok, unless an abridgment of the course for an addition of independent work be judged desirable, but believes that, because of the good effects of occasional change of pace in an arduous laboratory course, under the present limitations of time allotted to anatomy, the use of prepared dissections in moderation is pedagogically more sound than the practice of complete dissection for students of all degrees of maturity.

The prepared dissections are embalmed in a strong mixture which includes carbolic acid and arsenic and are kept under solution in small conveniently located cast-iron tanks lined with fire enamel. It is estimated that the parts will remain serviceable for five years, although Bangkok is within thirteen degrees of the equator.

It is claimed for the methods here set forth that in their entirety they prevent wastage of energy from duplication and misdirected effort and that they favor a clear grasp of the more fundamental facts of anatomy. It is believed that they fix in the student's mind more than do most schemes of anatomical instruction a synthesized view of the total anatomy of organs without prejudicing an appreciation of the subdivisions of anatomy as scientific units. They are adapted to the development of habits of self-activity, though they should perhaps be abridged to allow the addition of shorter courses in the fields especially interesting various members of the staff and designed to emphasize independent thought and action. It is believed that they are suitable for use with the most mature type of medical student.

STUDIES IN STAIN TECHNIQUE

II. CYTOLOGICAL STAINING WITH AUERBACH'S METHYL GREEN-ACID FUCHSIN MIXTURE

JAMES O. FOLEY

Department of Anatomy, The Tulane University of Louisiana

INTRODUCTION

In cytological procedures, especially those dealing with the germ cells, it is a well-known fact that in most cases Auerbach's stain gives very uncertain results or fails entirely. The failure of this mixture to give a good cytological stain appears to be due to the character of the fixative employed. The following method is the result of efforts to secure a mordant and develop a technique by which this stain may give satisfactory results, following fixatives to which it is ordinarily negative.

STAINS AND TISSUES EMPLOYED

A methyl green and an acid fuchsin dispensed by the National Anilin and Chemical Company were used in these investigations and gave excellent results.

The following stock solutions were used:

Methyl green (58 per cent, representing 0.116 gram actual dye), 0.2 gram per 100 cc. of distilled water
Acid fuchsin (65 per cent, representing 0.65 gram actual dye), 0.1 gram per 100 cc. of distilled water

The ready stain is prepared from the stock solution by adding 80 cc. of the methyl-green solution to 20 cc. of the acid-fuchsin solution.

The tissues used consisted of paraffin sections of the testes of the lubber grasshopper (Romalea microptera), the English sparrow (Passer domesticus), and the opossum (Didelphys virginiana). Some of these tissues were fixed in Gilson's, some in Allen's B15, and some in Flemming's strong fluid, as modified by Hance ('17, '25).

EXPERIMENTS

The technique employed by Bensley ('11) was used, previous to mordanting and staining, to remove any substances left in the tissue by the fixative which might interfere with staining. Sections of tissues, irrespective of the kind of fixing fluid used, were treated with a 1 per cent aqueous solution of potassium permanganate for five minutes, followed by treatment with a 1 per cent aqueous solution of oxalic acid for five minutes to remove the permanganate. Following treatment with oxalic acid, the sections were washed in running water for thirty minutes. The only exception to this rule was in the case of mercury-fixed material, which was first treated with iodine before the permanganate was applied.

Bensley (loc. cit.), in his studies on the pancreas of the guinea-pig, secured excellent differentiation of methyl green for basophilic structures and of acid fuchsin for acidophilic structures following fixation of tissue in an osmicated fluid. Although the stains in this case are applied separately, and not in a mixture such as Auerbach's, the positive results secured, following an osmicated fixing fluid, indicate that Auerbach's stain likewise should follow osmicated fixing fluids.

Flemming's strong fluid, especially the modification of Hance (loc. cit.), is not only a valuable fixative for many tissues, but practically all of the more important cytological stains seem to follow it better than any other fixing fluid. Sections of tissues which have been fixed in Flemming's fluid and stained with Auerbach's mixture, after Guyer, only occasionally gave good results. A good stain was obtained in the chromatic structures, but often the achromatic structures failed to take the acid fuchsin. The formula of Auerbach's stain as given in Lee did not give even satisfactory nuclear results. The acid fuchsin is in excess in this formula.

It would appear that mordanting with osmic acid is necessary for nuclear anilin dyes in tissues which have been fixed in non-osmicated fluids. It has been previously shown that tissues fixed in these fluids require mordanting with fluids containing osmic acid before they can be stained with safranin and gentian violet (Winiwarter, '23; Allen, '27; Foley, '29). Likewise, methyl green, also an anilin dye, stains only those tissues well which have been fixed in a fluid containing osmic acid (Foley, loc. cit.).

Allen's B15 is probably more universally used than any other one fixing fluid and for the majority of tissues it gives superior results. However, it has the serious defect of rendering tissues negative in their staining with such stains as Flemming's triple combination and Auerbach's mixture. Sections of Bouin-fixed material were mordanted in a 0.5 per cent aqueous solution of osmic acid for from twelve to twenty-four hours and then stained with the formula and after the method as given in Guyer. Here, as in the case of Flemming-fixed material, the chromatin was stained by the methyl green, but the achromatic structures and cell products failed to take the acid fuchsin. Control sections of non-mordanted material were treated by the same method and yielded negative results for both the chromatic and achromatic structures.

Following the suggestion in Lee, that acids intensify acid fuchsin in tissues, varying amounts of 10 per cent acetic acid were added to the formulae employed. This resulted in a staining of the achromatic structures with fuchsin, but interfered with the staining of the chromatic structures with methyl green. Similar results were obtained when such procedures were tried on Flemming-fixed material. By reducing the amount of acid fuchsin, a slightly better nuclear stain was obtained in Bouin- and Flemming-fixed material. However, the differentiation was far from satisfactory. The improved results indicated that not only must the acidity be controlled, but also that the measured amount of the methyl green should be in excess in the formula.

The effect of applying the acid following staining was tried. After treatment with permanganate and oxalic acid, sections of Bouin-fixed material were mordanted for from twelve to twenty-four hours in a 0.5 per cent aqueous solution of osmic acid, washed in running tap-water for thirty minutes, rinsed in distilled water, and stained for twenty-four hours in a neutral mixture of the two dyes. The stain was prepared by adding 80 cc. of a 0.116 per cent aqueous solution of methyl green to 20 cc. of a 0.65 per cent aqueous solution of acid fuchsin. After staining, each slide was handled separately. The excess stain was wiped from the slide and the section surface pressed very lightly on blotting-paper. The slide was then immersed in 95 per cent alcohol which had been acidulated by the addition of two drops of a concentrated (36 to 37 per cent) hydrochloric acid. Each slide was allowed to remain in acidulated alcohol for periods of from five to thirty seconds, or sometimes longer. The time necessary for differentiation in acid alcohol varied with the tissue used and the fixative employed. From acid alcohol the slide was transferred directly to carbol-xylol for a few seconds—just long enough to remove the alcohol—and was then transferred to xylol. As a result of this procedure, excellent results were obtained. Equally good results were obtained in Flemming-fixed material by this method.

Both Guyer and Lee state that Auerbach's stain works best after a bichloride-of-mercury fixative. Tissues fixed in Gilson's fluid and stained with the various formulae of Auerbach's mixture, as given in the above references, invariably gave very poor results. However, if material fixed with this fluid was first mordanted with osmic acid and then stained with Auerbach's mixture, after the method just outlined, good results were obtained.

It is necessary to observe certain precautions in handling this stain. All substances left in the tissue by the fixative which might interfere with staining should be completely removed from the sections before mordanting or staining. There should be a decided excess of methyl green over acid fuchsin in the stain formula. If the chromatin fails to stain satisfactorily following a reduction in the strength or amount of acid fuchsin, the slides are either being left too long in the acid alcohol or else the amount of acid in the alcohol is in excess and must be reduced. Care must be taken that none of the solutions used in this method be alkaline, as alkalinity interferes with staining. Most tap-water is slightly alkaline; therefore, sections which have been washed in tap-water following mordanting with osmic acid should be rinsed in distilled water before they are placed in the stain.

Chromatic structures, such as the prophase, metaphase, anaphase, and telophase chromosomes; chromatin nucleoli, late pachytene threads of the meiotic prophase, and densely packed chromatin of the developing spermatids, stain a brilliant green. The more loosely arranged chromatin of resting cells, and of the leptotene and early pachytene of the meiotic prophase, varies from a dark blue green to a purple color. Achromatic structures, such as plasmosomes, spindle fibers, and tails of spermatozoa, range from a light pink to a brick red. Fibrous connective tissue and muscle stain a very dark red color. This stain is especially differential for chromatin nucleoli and developing spermatozoa.

SUMMARY OF PROCEDURE

1. After the removal of paraffin, the sectioned tissues are run through graded alcohols to water. The slides with their affixed sections are placed in a 1 per cent aqueous solution of potassium permanganate for five minutes, following which they are treated with a 1 per cent aqueous solution of oxalic acid to remove the permanganate. This procedure is followed in all cases except for those tissues which have been fixed in a fluid containing mercury, in which case they are first treated with iodine before the permanganate is applied.
2. Wash in running water for thirty minutes. If the material is fixed in a fluid which does not contain osmic acid, mordant the sections for from twelve to twenty-four hours in a 0.5 per cent aqueous solution of osmic acid. This mordant-

ing, of course, is unnecessary if the tissue is fixed in a fluid containing osmic acid.

3. Wash for thirty minutes in running water, rinse in several changes of distilled water, and stain for twenty-four hours in a mixture of 80 cc. of a 0.116 per cent aqueous solution of methyl green and 20 cc. of a 0.65 per cent aqueous solution of acid fuchsin.

4. Following staining each slide is handled separately. The excess stain is wiped from the slide and the section surface is pressed on blotting-paper. Do not dry the sections, they should appear moist.

5. Following blotting, transfer the slides to acidulated 95 per cent alcohol for from five to thirty seconds, or longer, as determined by trial. At the end of this time they are transferred to carbol-xylol for a few seconds and thence into xylol, from which they are mounted in balsam.

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INTRAVASCULAR PHAGOCYTOSIS OF ERYTHROCYTES IN NECTURUS FOLLOWING PROLONGED IMMERSION IN LEAD ACETATE

ALDEN B. DAWSON
Zoölogical Laboratory, Harvard University

TWO PLATES (TEN FIGURES)

Although mononuclear macrophages may appear in relatively large numbers in the circulating blood of vertebrates under the influence of a variety of physiological and pathological stimuli, it is but rarely that intravascular phagocytosis of erythrocytes has been described, even when extensive destruction of such cells has been taking place in the blood stream. Injured, fragmented, and senile red blood cells are generally filtered from the blood by the spleen and the liver and there ingested; and the phagocytic cells with their erythrocytic inclusions very seldom appear in the peripheral circulation (Jordan and Speidel, '23; Speidel, '26).

Phagocytosis of erythrocytes within blood vessels was studied in the transparent tails of amphibian larvae by direct observation by Clark and Clark ('28). The ingestion of blood cells was observed to occur in capillaries in which the blood had been stagnant for some time and, in animals with abnormal erythrocytes, to even take place in the circulating blood itself. Pigmented, mononuclear wandering cells of the tissues (macrophages) were the active agents. Cells of this type were followed as they migrated through the vessel wall into the lumen, where they proceeded to engulf abnormal red blood cells. Since the literature on this topic has been so recently reviewed by Clark and Clark, no extended discussion of earlier observations seems necessary. References will be made only to such studies as bear directly on the problem under consideration.

The observations on Necturus, on which the present report is based, were made on a series of animals in which lead acetate had been used in an effort to modify the segregation apparatus of the erythrocyte (Dawson, '28 a). The most striking feature of the blood picture of the treated animals, however, was the presence of large numbers of macrophages containing varying numbers of ingested red blood cells.

MATERIAL AND METHODS

The animals used in this study were obtained from Meadville, Pennsylvania, in October, and held at a low temperature (5° to 9°C.) until needed for experimentation. They were then placed in separate tanks and kept at room temperature. In practice each animal was held in four liters of tap-water to which 20 cc. of 1.0 per cent solution of lead acetate were added, and the solution was changed every third day. The lead proved to be mildly irritating to the body surface, as was indicated by the increased slime production of the cutaneous glands. Some irritation of the digestive tract was also noted. Normal animals, held without feeding, ordinarily discharge a small number of mucous pellets, composed of a material resembling meconium. In the control animals such pellets were found only infrequently in the tanks and were light brown in color. In treated animals, however, the number was increased and the color changed progressively from light brown to yellow-green, to light green, to emerald green, and finally to a deep dark green.

The color of these pellets is largely due to the presence of bile pigment, although the type of algae in the water supply may also slightly influence the color of the contents of the digestive tract. Similar definite changes in bile color have been observed in amphibian larvae by Speidel ('26) following experimental hyperthyroidism which induced an increased rate of erythrocyte destruction; and in the present study the change in color of the mucous pellets was finally accepted as a good criterion of the occurrence of extensive phagocytosis of erythrocytes in the blood vessels of Necturus.

The blood examined was obtained both by aspiration from an afferent branchial artery and by cutting the tail. Supravital preparations, stained with neutral red alone or in combination with Janus green, as well as smears were studied. The various organs which are most probably involved in the production of the changed blood picture were also fixed and prepared for study and will be the subject of a later report.

DESCRIPTION

Ingested red cells may be observed in the blood stream as soon as five days after exposure to lead acetate, but it is not until the seventh to the tenth day that phagocytosis becomes pronounced. It usually reaches its maximum by the fifteenth day and may continue for long periods until the blood is greatly depleted of erythrocytes and becomes pale, thin, and watery.

The effects of the lead poisoning persist long after the animals are removed from the lead environment. In one animal, now under observation, treatment with lead was begun on the 23rd of November and discontinued on the 28th of December. Examination of the blood on January 21st showed, however, an occasional freshly ingested erythrocyte, but the majority of the macrophages contained only granular débris.

The cells mainly concerned in the ingestion of erythrocytes are similar in all respects to the monocytes of the blood (fig. 5). These cells have been studied in both supravital neutral red-Janus green preparations and in stained smears. This relationship between monocyte and macrophage is most clearly demonstrated in cells which contain but one freshly ingested erythrocyte (fig. 7). In such cells the cytological details, rosette of neutral-red vacuoles and mitochondrial distribution, are not obscured by great accumulations of engulfed material, but the presence of the single red blood cell unmistakably marks the cell as a differentiating macrophage.

These observations are in complete agreement with the conclusions of Lewis and Lewis ('26), who were able to follow in hanging-drop cultures of whole blood the transformation of monocytes into macrophages, epithelioid cells, and giant cells. However, as already pointed out, Clark and Clark ('28) found that in the tadpole pigmented, mononuclear, wandering cells of the tissues were the elements active in intravascular phagocytosis. Pigmented mononuclear cells are not normal constituents of the blood of Necturus and are usually only encountered in blood secured by cutting the skin. In such cases they doubtlessly come from the tissues. Pigmented cells are also absent from the blood of lead-treated animals in the early stages of erythrocyte destruction, but may become quite numerous in the later stages. Whether the pigment within these macrophages represents an end product of the digestion of ingested material or whether pigmented cells from the tissues have migrated into the blood vessels it is difficult to say.

The macrophages vary greatly in size and in the amount of ingested material. In blood obtained from the tail (fig. 1) the number of engulfed red cells is usually small, rarely exceeding five, but occasionally a cell with as high as ten erythrocytes included in its cytoplasm is encountered. The large macrophages were obtained in great numbers from the afferent branchial vessels (figs. 3, 4, 6). There is probably some concentration of these elements in this area, as the finer vessels of the gills would tend to act as filters and hold back large cells which do not pass readily through their lumina.

In the early stages of phagocytosis the relative ages of the macrophages can often be roughly estimated both by the number and freshness of the included erythrocytes as well as by the volume of their cytoplasm and the amount of pigment and granular débris present. The freshness of an ingested red cell is indicated by the avidity with which its cytoplasm and nucleus stain with neutral red, the staining increasing in intensity as digestion proceeds. In these apparently younger macrophages, which have engulfed large numbers of red cells, the cytoplasm often appears to be distended to its limit, so that in many places only a thin film covers the ingested cells. In making smears it is almost impossible to obtain these cells intact. They usually rupture and the partially digested contents are scattered about in their vicinity. As digestion proceeds the macrophages come to contain an increasing number of globules and granules of varying sizes and the volume of the cytoplasm is also markedly increased. Usually accumulations of brown or black pigment characterize such cells, but occasionally a cell is observed which is completely filled with highly refractile globules (probably fat) but contains neither pigment nor foreign material reacting with neutral red (fig. 9).

The freshly ingested red cells usually appear smaller than the surrounding free cells and frequently have a denser color. Much of the apparent change in size is due to the change in the shape rather than the volume of the cell, as the ingested cells are no longer flattened ovals, but are spherical. The deeper color of the hemoglobin may also be partially accounted for in this same way, but there also appears to be a gradual reduction in the volume of the cell as digestion proceeds, and this may be due to some process of dehydration, as suggested by Lewis and Lewis ('26), but no wrinkling of the surface membrane is discernible in these partially digested cells.

Coincident with the increase in the number of cells of the mononuclear series, there is also an increase in the number of the eosinophiles. In Necturus these cells are normally quite numerous, often equaling the neutrophiles in number. Ordinarily the nuclei of the eosinophiles are simply lobed, but in the treated animals many cells attained quite complex nuclear patterns. In smears the cytoplasm appears to be increased in volume and is frequently conspicuously vacuolated, and in several instances an ingested red blood cell or hemoglobiniferous fragments were observed (fig. 8). These observations on dried films were frequently confirmed in supravitally stained preparations. Similar observations have been made on eosinophilic granulocytes of the dogfish by Lewis and Lewis ('26), and Jordan and Speidel ('28) note as a specific feature of the subserous erythrocytophagic activity in the horned toad following intraperitoneal hemorrhage, the presence of a few phagocytic eosinophiles with hemoglobiniferous fragments. In view of the very striking migratory activity of these cells, it is not surprising to find them occasionally participating to a small degree in phagocytosis of fairly large objects. However, no evidence of similar activity of the neutrophiles was obtained.

The nature of the changes induced in the blood stream whereby monocytes attain the ability to ingest red blood cells is not clear. Obviously, the erythrocytes are injured by the exposure to the lead salt, but whether the substances lost from them directly stimulate the phagocytic capacities of the monocytes, or whether the monocytes ingest the red cells because chemical or physical changes in these render them more easily phagocytosed, is not apparent.

Clark and Clark ('28) noted that the presence of stagnant blood in newly formed capillaries exerted an attraction on the pigmented wandering cells of the tissue and that these phagocytes never ingested extravasated blood cells until the latter had remained at least fifteen hours in the tissues. That some 'aging' of the erythrocytes is necessary to phagocytosis is also indicated by the observations of Lewis and Lewis ('26) that the ingestion of red cells by transformed monocytes in cultures of whole blood did not take place until after at least twenty-four hours had elapsed. Clark and Clark ('28) also made the additional observation that abnormal erythrocytes in the circulating blood display a quality of stickiness, when they come in contact with macrophages within the vessels, that is not possessed by normal red cells. The abnormal cells, however, did not react in this way to endothelium or to the other formed elements of the blood.

Direct observations on blood freshly drawn from lead-treated animals reveal a significant number of erythrocytes that are characterized by a loss of typical form and a deeper color of the hemoglobin. Some of these cells are spherical, while others are rounded spindles. The more intense coloring with hemoglobin is due in part, if not entirely, to the loss of the characteristic, flattened oval form.

These modified red cells, in contrast to the normal cells, also tend to stick to the glass slide, and when subjected to manipulation by pressure on the cover-slip they frequently cannot be displaced, or if displaced, tend to 'tail out' as they move away from their original position. This acquisition of stickiness by the surface of the cells is also accompanied by a decrease in the viscosity of their contents, so that the nuclei are readily displaced within the cytoplasm by manipulation due to pressure on the cover-slip. Similar changes in the physical properties of red cells have also been previously demonstrated in erythrocytes held in plasma over long periods of time (Dawson, '28 b). It was not possible, however, to observe in sealed cover-slip preparations whether the abnormal erythrocytes displayed the quality of stickiness when approached by a macrophage, as the red cell was fixed to the glass, and not freely moving as in the circulating blood.

Changes in red cells which involve merely size and form are apparently of little significance in the blood of amphibians, although fragmentation seems to favor phagocytosis in the mammals. The evidence that form and size are not primarily significant so far as intravascular phagocytosis is concerned is found in the studies of Emmel ('24) on the blood of Batrachoseps. In these animals, erythroplastids of varying size and form constitute over 90 per cent of the hemoglobin-bearing elements and no evidence of increased phagocytosis was recorded. It is only when fragmentation is associated with other changes in the erythrocytes that this phenomenon apparently assumes importance in relation to phagocytosis.

From the evidence already presented it appears clear that a great number of the macrophages present in the blood arise from monocytes in the circulation by a process of hypertrophy and individual transformation. The source of the increased number of monocytes is not so evident. Lymphocytes were not greatly increased in number and transitional stages between lymphocytes and monocytes were not numerous. No intravascular mitosis was observed. Definite conclusions on this point accordingly must await a detailed study of the changes produced in the lymphomyeloid tissues of the experimental animals.

SUMMARY

Extensive phagocytosis of erythrocytes occurs in the circulating blood of Necturus following prolonged immersion in lead acetate.

Mononuclear phagocytes, arising chiefly by a transformation of blood monocytes, are the predominating agents in the earlier stages of the destruction of the red blood cells. A relatively few eosinophilic leucocytes were also observed with ingested fragments of erythrocytes. Later, pigmented mononuclear cells appeared. Whether they represent monocytic phagocytes containing the end products of the digestion of erythrocytes, or are other pigmented cells attracted to the blood vessels from the tissues by the changes occurring in the blood, was not determined.

The destruction of erythrocytes was accompanied by striking changes in the color of the bile, which could be followed directly in the living animal by noting the color changes in the pellets of meconium-like material excreted from the digestive tract.

Physical changes in the erythrocyte, involving loss of the typical form and the acquisition of the quality of stickiness (to glass), appear to favor phagocytosis. No evidence of fragmentation was noted. Entire erythrocytes were ingested.

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PLATE 1

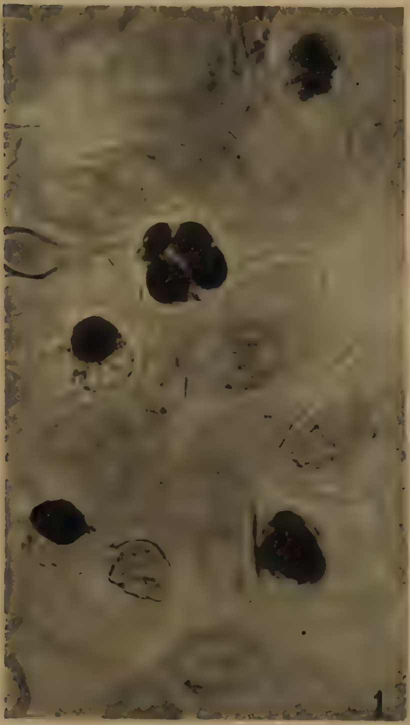
EXPLANATION OF FIGURES

1 Fresh blood from an incision in the tail, showing five macrophages. The ingested erythrocytes are stained intensely with netural red.

2 Fresh blood, aspirated from an afferent branchial artery, showing a single large macrophage whose contents are also stained with neutral red.

3 A large macrophage stained with neutral red and seen under higher magnification. A normal-sized monocyte is shown in the lower left-hand corner. Blood from branchial circulation.

4 A large unstained macrophage in a thick film of fresh blood. The nucleus is recognized with difficulty. The cell is greatly distended with engulfed material and in many places on its surface only the thinnest film of cytoplasm covers the ingested erythrocytes. Blood from branchial circulation.



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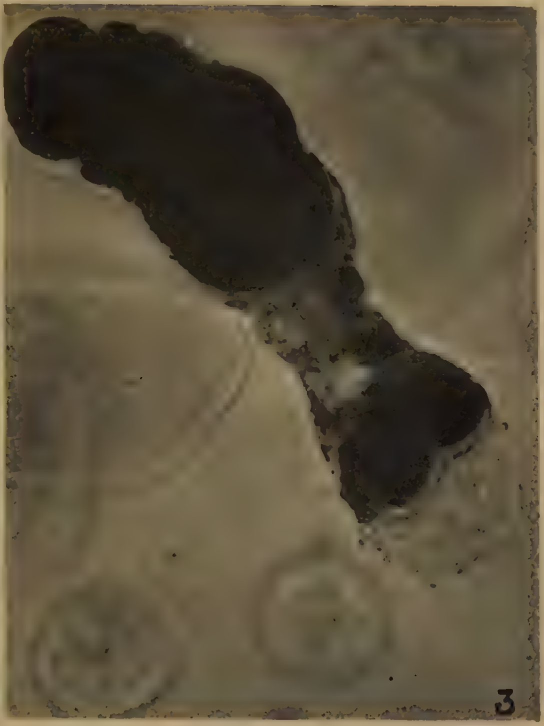

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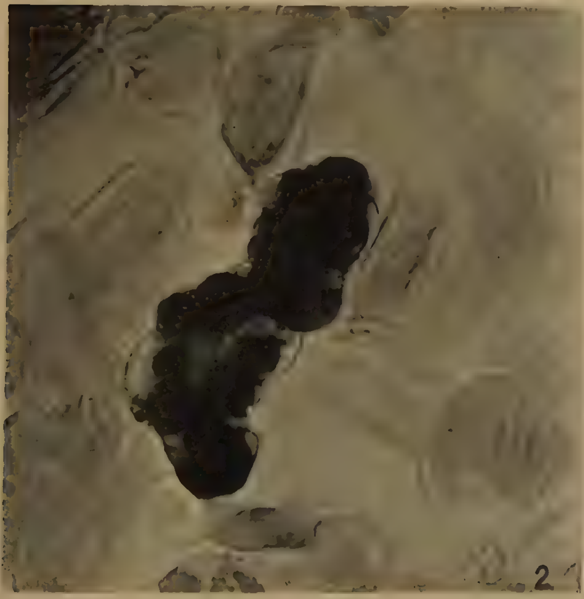

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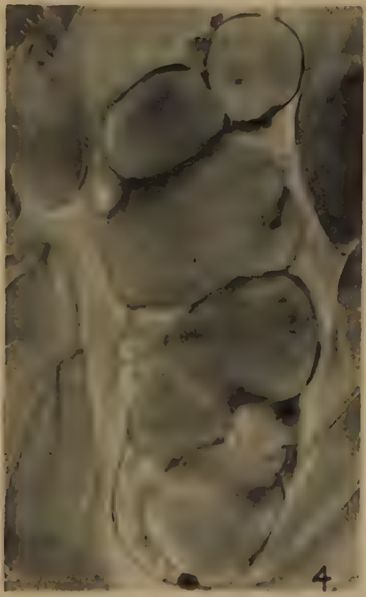

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PLATE 2

EXPLANATION OF FIGURES

5 A large monocyte showing the rosette of neutral-red granules.

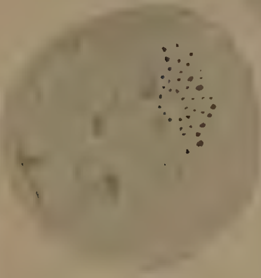
6 A large macrophage stained with neutral red, showing ingested red blood cells in varying stages of digestion together with granules and clumps of black and brown pigment. The cytoplasm is increased in amount and does not appear to be stretched over the inclusions, as in the younger macrophages. Blood is from the branchial artery.

7 A phagocyte showing one ingested erythrocyte and several other inclusions that do not stain with neutral red. A typical rosette, near the indented nucleus, is present.

8 An eosinophile containing three fragments of erythrocytes. Wright's blood stain.

9 A macrophage filled with highly refractile globules (possibly fat) that do not react with neutral red.

10 A macrophage filled with hemoglobiniferous fragments which stain intensely with neutral red. The larger fragments are grouped near the periphery of the cell.



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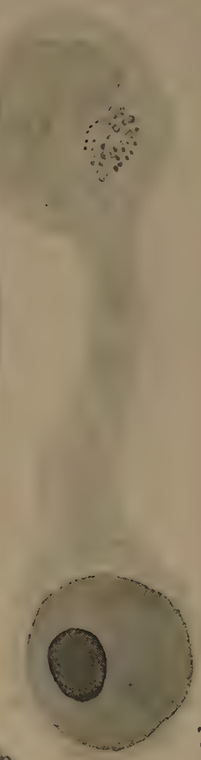

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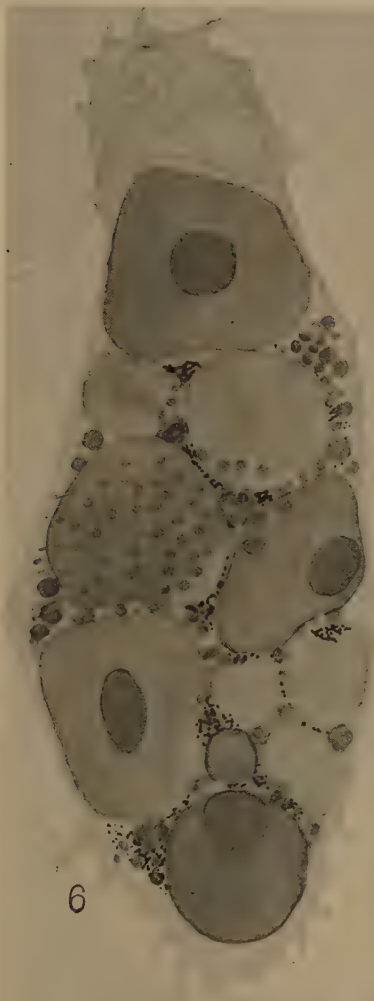

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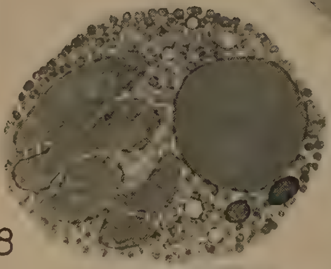

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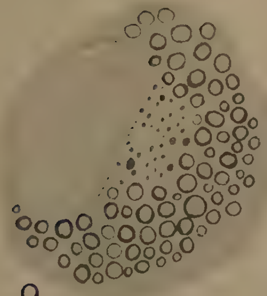

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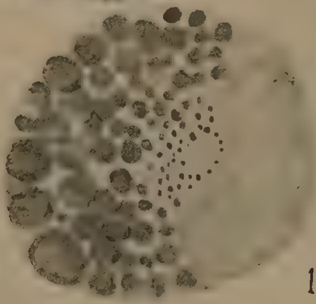

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BOOKRACK FOR McCLINTIC DISSECTING-TABLES

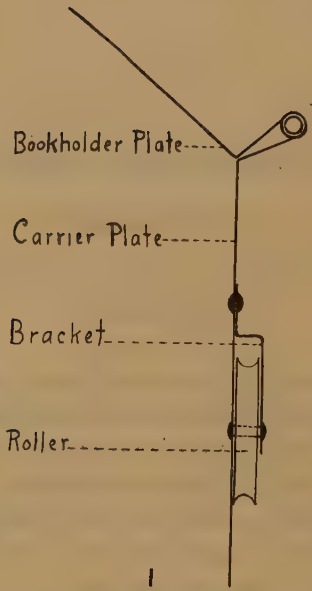
J. M. ESSENBERG
Anatomy Laboratories, University of Loyola Medical School, Chicago, Illinois

THREE FIGURES

The bookrack which is described here was designed by the writer while associated with the Anatomical Laboratories of the University of Oklahoma Medical School. It was produced to fill a gap in the dissecting-room equipment of that institution. Heretofore, students were obliged to place their books on the cadaver—which, besides soiling the books, is very awkward and inconvenient to the student. It was thought undesirable to install bookracks that are permanently attached to the frame of the dissecting-table. They interfere with the movements of students around the tables as well as with transportation of cadavers, especially in dissecting-rooms where space is limited. Permanently attached bookracks are also objectionable on account of the untidy appearance they impart to the dissecting-room.

CONSTRUCTION

Roughly speaking, the shape of the bookrack is that of a rectangular building in which the front and rear sides are omitted and in which the sloping roof serves as a book-holding plate (figs. 1 and 3). It is made of 20-gauge sheet iron and measures 14 inches in length, which is long enough to support an opened text-book or atlas. It consists of four main parts: two book-holder plates and two carrier plates. The former are 13 inches in width and are hinged at the top. The ends are rolled downward, for the sake of reenforcement. The carrier plates are hinged to the book-holder plates in


Bookholder Plate
Carrier Plate
Bracket
Roller

1

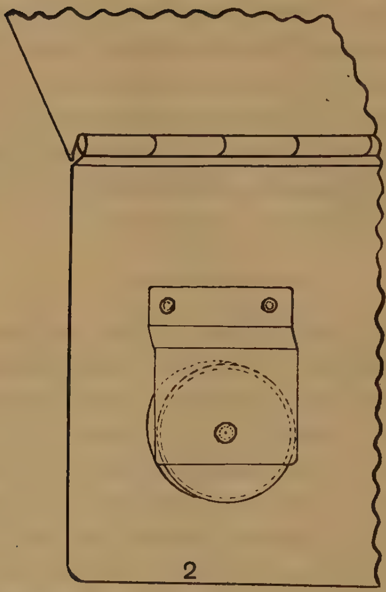


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Fig. 1 Partial end view, showing attachment of roller.
Fig. 2 Partial side view, showing hinge and roller.

such a fashion as to produce a ledge which supports the book. The manner in which this is made is best understood from figure 1. The ledge, including the hinge, is ⅞ inch wide and is formed by both book-holding and carrier plates. It makes a right angle with the former and an angle approximately 135° with the latter. All of the hinges are of the continuous type (fig. 2), and the interdigitations are ¾ inch wide and well fitted together, so as to afford neatness in appearance and smoothness of action. The pins are 3/16 inch in diameter and are slightly riveted at both ends so as to keep them in place.

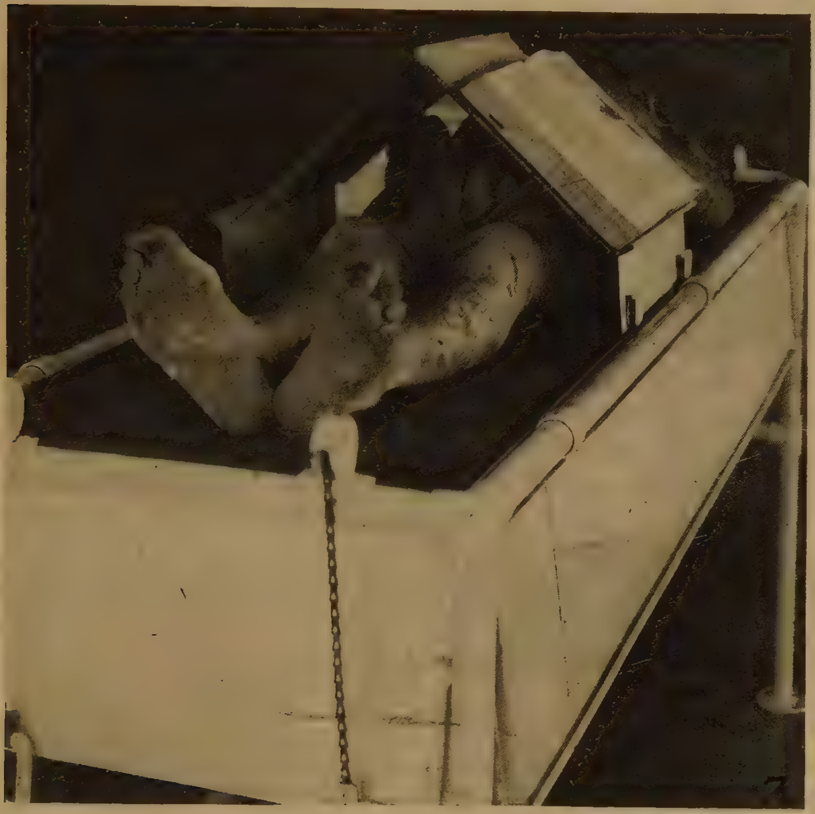


Figure 3

Excluding that part of the carrier plates which goes to make the ledge, they are 6 inches in width and, like the book-holder plates, 14 inches in length. They are not reenforced in any way and the free corners are cut off or rounded to facilitate freedom of motion. Each plate is provided with two ordinary pulley castors measuring ¼ inch in thickness and 1½ inches in diameter. It is best to see that the groove of the roller is deep (⅛ inch) and the edges sharp; this adds to the stability of the bookrack. The castors are attached to the carrier plates by brackets in a manner shown in figures 1 and 2. It must be noticed that that part of the bracket which overlies the roller is made as short as possible, being just long enough to allow proper attachment of the pin. Of course, the roller must play freely upon the pin. They are set as far as possible apart and approximately 3 inches from center of the roller pin to bottom of carrier plate.

In constructing the bookrack the following dimensions must be observed. The angle between the book-holding plates must be approximately 100° and that between the book-holding plates and the carrier plates about 130°. The height of the bookrack from center of upper hinge to lower edge of carrier plate is 14¼ inches. The distance between the two carrier plates must not be less than 20 inches; although the top of the table is 19¾ inches wide, ¼ inch is allowed for spring power, which insures better contact.

OPERATION

As seen from figure 3, the bookrack rests on the free edge of the pan. It can be rolled from one end of the table to the other without being interfered with by the cadaver or any part of the table. It supports one book on each side in a position which is equally good for the student whether in the standing or the sitting position. At the end of the dissecting period, the bookrack is folded much in the fashion of closing a book and is placed on the cadaver to be covered by the lid. In folding, the book-holding surfaces are covered and cannot be soiled. It is light in weight (less than 7 pounds) and can be easily handled. It is white-enameled, which makes it neat in appearance.

This bookrack has been designed for use on the McClintic dissecting-tables, but it can be used with equal facility on the Kornhauser-Johnson tables. The pan in the latter table is slightly wider and this will necessitate a proportional increase in the width of the book-holding plates. The dimensions of the carrier plates need not be changed.

MANUFACTURER AND PRICE

The bookrack was manufactured by the Henderson Brothers Sheet Metal Works, 327 West Second Street, Oklahoma City, Oklahoma. The price paid was $6.00 apiece. The local manufacturers took special interest in the production of this rack. They gave lower prices and better workmanship than any of the many manufacturers consulted.

A CASE OF HUMAN RIGHT AORTA

DAVID H. SPRONG, JR., AND NORMAN L. CUTLER
Department of Anatomy, Johns Hopkins University

THREE FIGURES

The present paper describes a case of right aorta and offers a review of one hundred additional cases from the literature, in an attempt to discover any significant statistical data, concerning frequency, sex ratios, age, and associated abnormalities.

DESCRIPTION OF NEW CASE

The case here reported is that of a white male fifty to sixty years old. The cadaver had been used for a student dissection and had been almost completely dissected before the present description was undertaken. The specimen consists of the trunk, arms, and thighs, all thoroughly dissected. The anterior body wall, together with most of the viscera, has been removed. Enough remains, however, to establish the following points:

The heart is normal, but crowded somewhat to the left. The great veins and pulmonary artery are normal in position. The aorta arises normally, but arches to the right and backward over the right bronchus and continues caudally slightly to the right of the midline. The branches of the arch appear in reverse order: left innominate, right carotid, right subclavian. The ligamentum arteriosum is about 1 cm. long, arising from the right pulmonary artery about 4 mm. from the pulmonary bifurcation and joining the aorta about 6 mm. distal to the apex of the arch. The junctions with the ligamentum are marked inside the pulmonary artery and aorta by small, wrinkled depressions.

The relations of the vagus nerves show complete reversal, the right passing in front of the arch of the aorta. The right recurrent laryngeal nerve loops around the aorta and the left around the left subclavian artery.

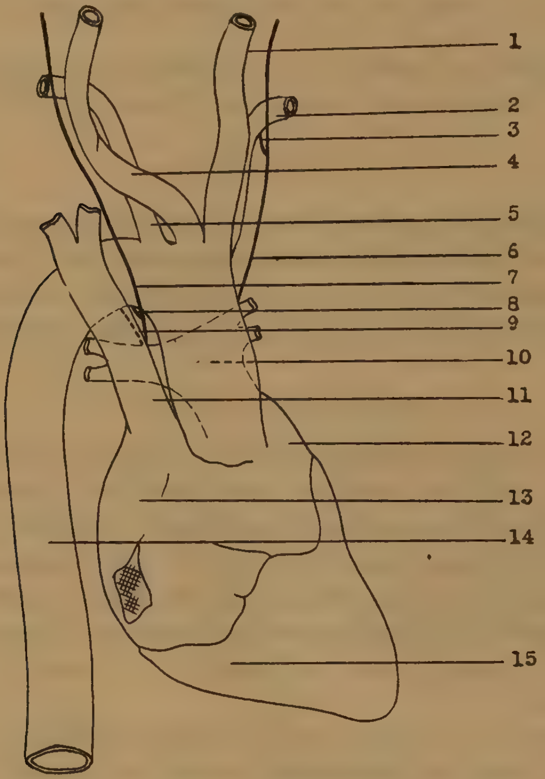
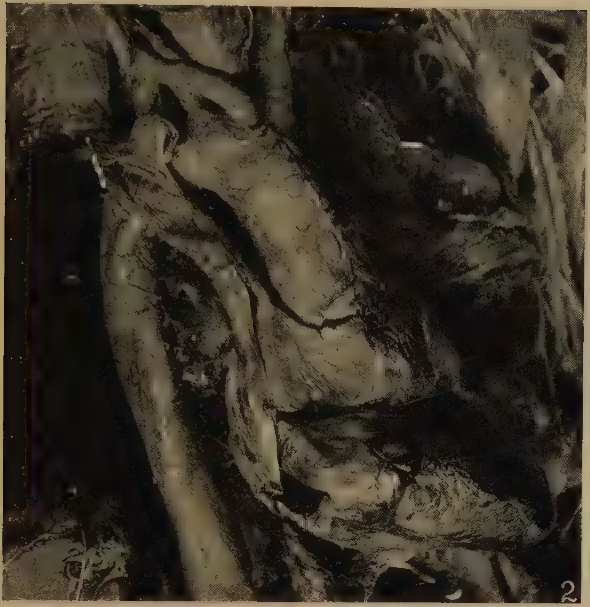


Fig. 1 Heart and great vessels from in front: *1,* left common carotid artery; *2,* left subclavian artery; *3,* left recurrent laryngeal nerve; *4,* right common carotid artery; *5,* right subclavian artery; *6,* left vagus nerve; *7,* right vagus nerve; *8,* right recurrent laryngeal nerve; *9,* ligamentum arteriosum; *10,* pulmonary artery; *11,* superior vena cava; *12,* left auricle; *13,* right auricle; *14,* descending aorta; *15,* right ventricle.

The upper part of the thoracic duct, together with the jugular veins, has been removed. However, the part of the duct caudal to the fifth thoracic vertebra is in this case situated distinctly to the left of the midline rather than, as normally, to the right.



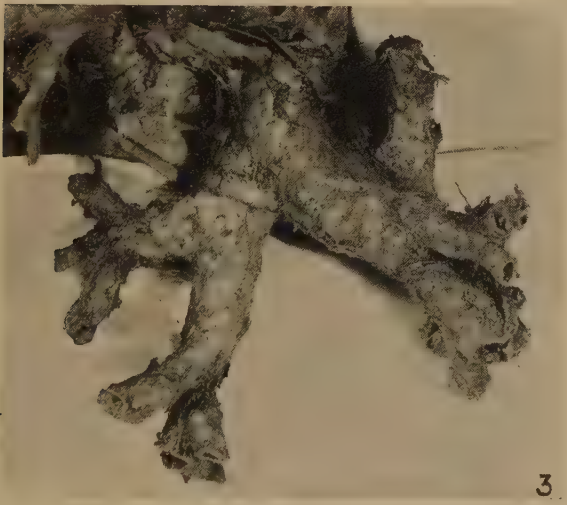


Fig. 2 Photograph of heart and great vessels in case described, ventral view.
Fig. 3 Photograph of trachea and bronchi, ventral view.

Unfortunately, the lungs were completely removed, so that their lobulation could not be studied. Certainly there was no marked symmetry reversal, or it would have been noted during the original dissection. However, the bronchi, which are intact, show an interesting abnormality. The tracheal bifurcation is normal and the two chief bronchi pursue the normal directions, but the relative diameters of the two bronchi are reversed, the left being slightly the larger. The eparterial bronchus arises normally from the right bronchus, which continues on and branches in the normal manner. The left bronchus, however, gives off a branch corresponding in position and direction to the eparterial bronchus and then continues on as the normal pattern of branches. The relations of the two bronchi and their branches are shown in figure 3. The significance of this condition is obscure. The relative sizes of the two bronchi and the presence of the left eparterial bronchus indicate symmetry reversal, but the directions of the bronchi and the presence of the right eparterial bronchus are normal.

LITERATURE

For discussion, the cases of right aorta will be considered in the following groups, according to the amount of transposition and abnormal branching associated with them: A) Situs inversus viscerum. B) Right aorta, without inversion of other viscera: *a*) Double aorta. *b*) Right aorta, giving off left carotid, right carotid, right subclavian, left subclavian. *c*) Right aorta, giving off left innominate, right carotid, right subclavian.

Situs inversus viscerum

By far the commonest type of abnormality described in connection with right aortae is a complete inversion of the thoracic and abdominal viscera, so that they constitute a mirror image of the normal situs solitus. The details have been so often recorded that further description is unnecessary here. Situs inversus is frequently discovered clinically, since it is immediately suggested by the finding of a right heart or stomach, or a liver on the left. Diagnoses have often been confirmed, or even made entirely, by x-ray.

Many of the clinical descriptions, however, are so incomplete as to leave doubt concerning the true nature of the case. Examples of partial inversion of the viscera are so common that a diagnosis of situs inversus cannot be based solely on the finding of a right heart or stomach. The following cases found in the literature are believed to be authentic examples of situs inversus viscerum:

Anselmi, 1888; Baillie, 1788; Barbo, 1900; Beck, K., 1899; Burghart, 1897; Carpenter, 1918; Chaplin, 1854; Clapp, 1850; Crombie, 1917; De Bary, 1864; Englemann, 1919; Falcon, 1838; Griffith, 1891–1892; Grisolle, 1834; Grothe, 1898; Gruber, 1846 and 1865; Hartz, 1916; Heiman, 1919; Koller, 1899; Martland, 1916; McCrae, 1905; Moncreiff, 1828; Moreau, 1918; Morrill, 1919; McWhinnie, 1840; Naucler, 1916; Plate, 1898; Rahman, 1917; Ratkowsky, 1899; Schule, 1875; Schulte, 1918; Smith, 1919; Snowden, 1836; Thane, 1888; Upson, 1918; Virchow, 1861; Watson, 1836.

Double aorta

Fifteen cases of double aorta were found. With slight variations, the condition is as follows: the heart is normal; the ascending aorta arches slightly to the right and bifurcates to form: *a*) A large right arch, which passes backward over the right bronchus, and after giving off the right carotid and subclavian, arches to the left behind the esophagus; *b*) a smaller left arch, which passes in front and to the left of the trachea, giving off the left carotid and subclavian, and unites with the right arch, behind and to the left of the esophagus, to form the descending aorta.

In two cases only (Buschendorff, '17; Neddersen, '02) the left arch is the larger of the two. In five cases (Arkin, '26; Brigham, '22; Thomson, '63; Turner, '62; Watson, '77) the posterior part of the left arch (from the left subclavian to the descending aorta) remains only as an obliterated vessel or fibrous cord. These observations seem to indicate that the usual condition of double aorta represents primarily a right aortic arch with a secondary persistence of the left arch.

No associated anomalies were described. In only one instance (Hermann, '28) was any clinical manifestation mentioned. In this case the two arches caused constriction of the esophagus and trachea, giving rise to dysphagia and also to cyanosis after exercise. This latter symptom was doubtless partly accounted for by the patent ductus which was present.

Curnow, 1875; Dittrich, 1886; Ewald, 1926; Hamdi, 1906; Heller, 1904; Kajava, 1914; Shaw, 1897.

Right aorta, giving off left carotid, right carotid, right subclavian, and left subclavian

Aside from total situs inversus, this group, comprising twenty-six cases, is the largest found. It is interesting to note that the homologous condition of terminal right subclavian is one of the commonest anomalies found in connection with the usual left arch.

In these cases the aorta arches to the right and backward, over the right bronchus, giving off in order the left carotid, right carotid, right subclavian, and finally the left subclavian, which passes to the left behind the esophagus. The ligamentum arteriosum passes to the left of the trachea and esophagus and joins the left subclavian. A slight variation of this pattern is described in seven cases (Dittrich, '86, two cases; Fioratti and Aglietti, 1763; Gruber, '63; Herringham, '91; Quain, '44; Turner, '62). The upper part of the descending aorta presents behind the esophagus a blind pouch, which gives rise to the left subclavian and to which the ligamentum is attached.

Associated anomalies are rare in this group. In only two cases (Meckel, 1809; Dickson and Fraser, '13–'14) is a defective septum present. In Meckel's case there was also an intestinal diverticulum. In two cases (Hurley and Coates, '27; Mutel and Fourche, '20) both right and left superior venae cavae were present. In one case (Herringham, '91) death was caused by constriction of the esophagus and trachea.

Abbott, 1892, Dévé, 1925; Ewald, 1926; Ghon, 1908; Gottschau, 1887; Hammer, 1926; Klinkosch, 1776; Lockwood, 1884; Lockwood and Withers, 1890; Renander, 1926; Riche, 1897.

Right aorta, giving off left innominate, right carotid, and right subclavian

In this type of anomaly there is an exact inversion, or symmetry reversal of the aorta and its branches, but no inversion of other viscera. Thirteen cases of this kind were found. A striking feature of this group is the large percentage associated with an abnormal heart. In one case (Turner, '83) there is only a single auricle and a single ventricle. The pulmonary artery is fed through the ductus. In three cases (Bracht, '08; Cailliot, 1807; Obet, 1808) the septum ventriculorum is imperfect and the aorta communicates with both ventricles. One case (Abernethy, 1793) is associated with a right heart.

From the arrangement of the aorta and its branches, the new case described above falls in this group. However, in none of these cases was the presence of a left eparterial bronchus or a left thoracic duct mentioned.

In the following cases no associated anomalies are recorded:

Bernard, 1818; Bosc, 1929; Ewald, 1926; Ewen, 1840; Panas, 1857; Stokes, 1909; Valleix, 1834; Vincenzi, 1885.

DISCUSSION

The new case described in the present paper is the only example of right aorta seen in 223 recent dissections at the Johns Hopkins Medical School. A number of authors have stated the number of cadavers dissected in finding one right aorta:

Bankart, Pye-Smith, and Phillips,	1 in 158
Barbo,	1 in 676
Brigham,	1 in 1400
Quain,	1 in 1040

Smith ('19) quotes Le Wald as saying that he has seen nineteen cases of complete situs inversus of thorax and abdomen. He estimates that there is one case in 3000 population.

Only very general inferences can be drawn from these figures regarding the frequency of right aorta, since in studies of the anomalies found in large groups of cadavers several authors have found no examples of right aorta at all.

The ages are fairly evenly distributed, ranging from seven months' gestation to eighty-four years. This is to be expected, in view of the fact that no clinical significance, except in the very few cases mentioned above, seemed to be attached to these conditions. Of the one hundred cases studied, fifty are males, twenty-eight are females, and in twenty-two the sex is not reported. This is practically a two-to-one ratio of males to females—due, doubtless, to the preponderance of males used for dissection.

A great deal has been said regarding the embryology of these conditions. The structures concerned are just the reverse of those in normal embryology. Newman ('23) considers the symmetry reversal either of a whole organism or of any part of it as due to monozygotic twinning. This theory offers a plausible explanation of situs inversus, but it is scarcely tenable in the case of right aorta without situs inversus. Here we have not the production of a new structure, but rather the failure to disappear of a structure which is already present in the embryo. The aortic arches are fundamentally paired structures, the right and left sets being mirror images of each other, and the adult condition of a single arch is a secondary arrangement brought about by the disappearance of one of the basic lateral components of the primitive aortic pattern.

It has been suggested that the persistence or disappearance of the arches is governed by mechanical factors during embryonic development. Comparative embryology offers a favorable approach to the study of this question, since the normal development of right aorta and left aorta can be followed and compared in the birds and mammals, respectively. Congdon and Wang ('26) have compared the arch development in the chick and pig, and point out that in the chick there is a greater cervical flexure and a greater displacement of the pulmonary trunk, so that the left aortic arch is compressed between the pulmonary artery and the ventral body wall, and is thus obliterated. In the pig, on the other hand, these factors do not operate, and since the original direction of flow from the conus is more to the right, the right arch grows at the expense of the left.

The possibility is immediately suggested that some such mechanical factors as those seen in the chick may play a part in the formation of the anomalous right aorta, or double aorta in man. A pronounced cervical flexure, together with the normal rotation of the aortic and pulmonary trunk, might well bring about enough pressure on the left fourth arch to cause its obliteration, or at least to make the competition between the right and left arches an even one.

RÉSUMÉ

A case of right aorta in a white male, fifty to sixty years of age, is described. The heart is normal. The branches of the aortic arch are just the reverse of normal. The relative sizes of the right and left bronchi are reversed, and both right and left eparterial bronchi are present. The thoracic duct is on the left. There is no inversion of the other structures.

One hundred cases of right aorta found in the literature are discussed from the standpoint of frequency, associated anomalies, and embryology. It is pointed out that the aortic arches are primarily paired structures, and that therefore a right or double aorta does not represent a case of partial twinning, as suggested by Newman. Mechanical factors operative during the late branchial stage are discussed as possibly responsible for these conditions.

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MITOCHONDRIA IN THE SUBMAXILLARY GLAND OF THE RABBIT, WITH PARTICULAR REFERENCE TO NERVE STIMULATION

H. C. MOLOY AND I. H. SMITH

Department of Pathology and Bacteriology, University of Western Ontario Medical School, London, Canada

THREE PLATES (FIVE FIGURES)

INTRODUCTION

The object of this paper is to report changes in the morphology of mitochondria in the submaxillary gland of the rabbit following stimulation of the chorda tympani and sympathetic nerves. As a preliminary part of the work, the mitochondria in the normal or resting submaxillary gland were studied. Particular attention was paid to the morphology of mitochondria in relation to definite time limits of gland stimulation. It is a study which brings up again the important question, as yet unsolved, of the rôle of mitochondria in cellular activity and in the formation of secretion granules.

MATERIAL AND METHODS

Male rabbits were utilized. Ether was used as the anaesthetic for the first ten animals. For the remainder of the experiments the stimulating action of ether was at least partially overcome by the intraperitoneal injection of 18 to 22 cc. of 10 per cent solution of urethane. This method gave good surgical anaesthesia.

The chorda tympani or sympathetic nerves were exposed and stimulated by a faradic current, the strength of which was just perceptible on the tip of the tongue. Fifteen such experiments were performed in which the chorda tympani was stimulated for five to thirty minutes. Four experiments were performed in which the sympathetic trunk on one side was isolated and stimulated by an interrupted faradic current for a period of thirty minutes to one hour. The opposite gland was always exposed along with the stimulated gland throughout the experiment and served as the control.

Blocks of tissue not exceeding 4 mm. in size were immediately removed from the stimulated and the control gland. The tissue was fixed and stained by Cowdry's modification ('18) of Regaud's fixation and Altmann's acid fuchsin-methyl green stain.

OBSERVATION

The normal gland

Briefly, the histological components of the submaxillary gland of the rabbit consist of two cell types, together with their ducts. Bensley ('08) designated the so-called clear cells which stain metachromatically 'tropochrome cells' and the type failing to take the stain, 'homeochrome cells.' The tropochrome cells make up the greater part of the gland. The homeochrome cells are interposed between the intralobular ducts and the other type. In the resting stage they are closely packed with serozymogen granules which are stained deep red by the mitochondrial technique used.

Mitochondria of the tropochrome cells. The mitochondria of the tropochrome cells (fig. 1), staining a brilliant red, stand out distinctively against the delicate green background of the cytoplasm. They are quite numerous, and the majority occur as delicate filaments. Occasionally, slender curved rods predominate. The mitochondria situated in the distal portion of the cytoplasm may show regular swellings along the length of the filaments, simulating cocci in chains. In this region, where the cytoplasm fades as the lumen of the acinus is approached, globoid forms may be seen. Many of these globoid forms prove to be filaments cut obliquely. Others are definitely globoid in shape. Filaments with bleb-like swellings at one terminal are occasionally seen. The characteristic form is the delicate filament. They tend to be scattered more or less diffusely through the cytoplasm. This is in accord with Rikita Honda's ('27) observation on the position of the mitochondria in the serous cell of the rat. We notice that the mitochondria in many acini show a tendency to cluster around the nuclei toward the basal portion of the cell, but not to the same degree as in the homeochrome cell.

Mitochondria of the homeochrome cells. The presence of large and small serozymogen granules in the homeochrome cells makes it difficult to study the mitochondria. With care, delicate mitochondrial filaments are discerned between the granules (fig. 3). They are more concentrated near the basement membrane, as Honda also observes in the serozymogen cell of the rat. However, a few mitochondria are present in the distal portion of the cytoplasm, but, when present, they are not so filamentous—rather more rod-like or definitely globoid in shape. In size and shape the filaments are similar to those in the tropochrome cells. In the resting stage the serozymogen granules are usually large and spherical in shape. These are useful points in distinguishing them from the smaller mitochondria.

The mitochondria of the duct cells are present in the form of extremely delicate rods and filaments (fig. 5). They are concentrated about the nuclei. The peripheral cytoplasm is free. Occasionally, a duct may be seen joining a homeochrome acinus. At the point of union the cells may contain serozymogen granules. The mitochondria of this secretory duct cell still retain the size and position which they characteristically maintain in the non-secretory ducts. In contrast to the intercalary ducts, the mitochondria of the larger collecting ducts are very numerous and chain-like in appearance, as described by Honda.

The stimulated gland

Mitochondria of the tropochrome cell. In the tropochrome cell following stimulation of the chorda tympani for five minutes, no change is noticed when compared to the control or normal gland. The mitochondria are spread diffusely through the cytoplasm and are filamentous in form. After ten minutes, there is a slight shortening of the filaments and they appear to be thicker, but the change is very slight. Further, certain experiments show an inclination on the part of the mitochondria to assume a radial arrangement, at right angles to the base of the cell. This is not noticeable in the control gland. The most marked change is noticed after twenty minutes' stimulation. Globoid forms are very numerous (fig. 2), the majority of the mitochondria having this form. None of the delicate filamentous forms noticed in the normal cell are present. Near the base of the cell the mitochondria have assumed the form of short thick rods. The contrast between the type of mitochondria in the control gland and in the stimulated gland is remarkable and can be best appreciated by comparing figures 1 and 2. Even after intensive stimulation for twenty minutes, although the form has changed, there appears to be no variation in the relative number of mitochondria. Following thirty minutes' stimulation, the characteristic form is the short thick rod. The mitochondria are scattered throughout the cytoplasm. Very few globoid forms and certainly none of the filamentous type of mitochondria are noticed.

Mitochondria of the homeochrome cell. The mitochondria of the homeochrome cells tend to show a similar morphological variation following stimulation of the chorda tympani. The disappearance of the serozymogen granules, as a result of physiological stimulation of the cell, clears the cytoplasm and the mitochondria are more easily discerned. Very little if any change is noticed following five or ten minutes' stimulation. The most marked change of the mitochondria in this type of cell also is noticed following twenty minutes' stimulation of the chorda tympani. An estimate of the change observed can best be gained by a comparison of figure 3 and figure 4. Figure 3 shows the filamentous mitochondria of the control gland entwining between the serozymogen granules. They are more numerous in the proximal portion of the cell around the nucleus. Occasionally, a few rod-like forms can be seen in the distal portion of the cell near the lumen. Figure 4 illustrates the effect of twenty minutes' stimulation. The serozymogen granules have disappeared and the mitochondria are present in the form of small globoid forms. Near the basement membrane a few rod-like forms are present. Occasionally, sections show an apparent decrease in number of mitochondria following stimulation, but the majority appear to show no numerical change. One gains an approximate estimate of the normal from a study of many sections. Many exhausted acini show rod-like forms in the peripheral cytoplasm. This indicates that, although the rod-like forms are shorter and thicker than in the control, there is a gradation in nearly every section examined from rod-like to globoid forms.

Thirty minutes' stimulation results in a picture very similar to that noticed in the tropochrome cells. Many acini show serozymogen granules present and the mitochondria conform more to the rod-like type.

The mitochondria of the intercalary and so-called secretory ducts appear to show no change in the stimulated gland (fig. 5).

Sympathetic stimulation

Mitochondria of the tropochrome cells. The sympathetic trunk was stimulated for intervals of thirty minutes to one hour. The mitochondria of the tropochrome cells, in size and shape, are similar to those seen in the control. The filamentous type predominates. From a survey of numerous sections one finds that the filaments are more concentrated at the basement membrane near the nuclei. They also tend to assume a radial arrangement at right angles to the basement membrane. The distal cytoplasm may contain fewer mitochondria. However, the change in size, shape, and position of mitochondria is not striking enough to definitely state that changes have occurred.

Mitochondria of the homeochrome cells. The cells of the homeochrome acini are filled to capacity by the serozymogen granules. This observation is in agreement with Stormont ('04) in that the sympathetic does not innervate the homeochrome cells. The mitochondria can be seen as delicate filaments between the granules and show no morphological change as a result of sympathetic stimulation. The mitochondria of the intercalary ducts show no definite change.

DISCUSSION

We find that after five minutes' stimulation of the chorda tympani nerve, changes in mitochondria have occurred which are very slight and appear to consist of a more orderly arrangement within the cell; a tendency to stream out in the cytoplasm at right angles to the basement membrane. Later, following ten minutes' stimulation, the filaments shorten and become thicker and more rod-like in form. At twenty minutes, the maximum change occurs. The mitochondria in both the tropochrome and homeochrome cells have become definitely globoid in shape. In certain sections it is impossible to find either the filaments or rod-like forms. Other sections removed at this stage show the cells of certain acini to be exhausted of granules and to contain mitochondria of the rod-like type. Following thirty minutes' stimulation, very few globoid forms can be discerned. The majority are in the form of short thick rods. Such changes have occurred in a gland stimulated to physiological activity by means of excitation of the nerve supply. Although it is necessary to admit, as a result of the great congestion in the gland, that such changes may be attributed to injury to the cells, i.e., cellular asphyxia or alterations in the water content, we assume until proved otherwise that they are part of the complex of reactions concerned in the elaboration of secretion.

Such men as Müller ('92), Hoven ('12), Regaud and Mawas ('09), Noll and Sokoloff ('05), and others have paid particular attention to variations in morphology of mitochondria. They more or less agree that mitochondria show changes in morphology, related in time to changes in the granular content of the cell. Certainly the morphology of mitochondria varies during the different stages of cell activity, but results concerning time relationships are conflicting. Diagrams from the work of Noll and Sokoloff show 'granular' mitochondria in the gastric mucosa of a fasting dog. At the beginning of the tenth hour of digestion the mitochondria have become filamentous. Ma ('28) shows bulbous swellings along the filaments five hours after feeding. In such an organ as the stomach it is difficult to associate such changes with a definite stage in the cycle of secretion. Champy ('11–'12) observes that in the intestinal epithelium during digestion and absorption the mitochondria become less numerous and less thread-like. Seecof ('25) notices an increase in the length of mitochondrial filaments in thyroid glands exhibiting compensatory hyperplasia. With increasing activity in the thyroid gland, Cramer and Ludford ('26) find the mitochondria to be larger and more filamentous. With diminished activity, their differentiation from the cytoplasm becomes less distinct and fewer granules are visible. R. R. Bensley ('11) studied with great care the granular acinar cells in the pancreas of the guinea-pig. The granular nature of such cells had suggested that they might be transitions between acinar and islet cells. In discussing the origin of the granules he describes an associated change in the mitochondria. He states that "associated with the disappearance of the basophilic material there is a profound modification of the mitochondrial filaments. When the base of the cell is well filled with tiny granules but some zymogen is still present, the mitochondria of the affected cells are seen to differ from the surrounding normal cells in the fact that the rods are shorter and thicker and show a tendency to round up in granules." Is it possible that this represents merely a stage in the functional activity of the cell or acinus and that an acinus adjacent to it could be in an earlier or a more advanced stage and the mitochondria assume a form characteristic of such a stage? Krogh ('22) mentions the great variation, locally and generally, in the capillary supply to resting and active muscle. One is lead to believe that such physiological factors may be applied to the internal glands in such a manner that under certain conditions gland cells may function in relays and so explain the variations in secretory stages mentioned above. Opie ('28) makes mention of this possibility and states that "when animals are fed at short intervals, part of the gland [pancreas] will be in one stage of secretion and part consisting of groups of acini will be in another."

On the other hand, Key ('16) finds no diminution of mitochondria or change in structure of the filaments during the process of secretion in the toad's pancreas, activated by secretin or by pilocarpine. Yet in relation to the submaxillary gland of the rabbit it is apparent that changes have occurred of such a nature as to merit consideration.

Concerning the relationship of such changes to the elaboration of secretion granules, we consider that Ma ('28) has very concisely described the modern conception. He states that "any one of the cytoplasmic constituents, mitochondria, chromophile substance or Golgi apparatus cannot be considered as the exclusive parent substance of the zymogen granules, and that the possibility of the simple type of transformation of mitochondria into zymogen granules by segmentation is completely excluded." Whether the globoid forms originate by segmentation of a filament or from a single filament is difficult to determine. Although certain exhausted acini appear to show a decrease in number of mitochondria, the majority appear to show no change. There seems to be a missing link in the cycle of events which should bridge the gap between the globoid form of mitochondria and the actual secretion granules.

Stormont ('28), as a result of nerve degeneration and stimulation experiments, claims that the chorda tympani nerve is secretory to the homeochrome cells and that the tropochrome cells display changes following stimulation of the cervical sympathetic. In agreement with him, we find that sympathetic stimulation fails to dislodge the serozymogen granules of the homeochrome cells. But similar stimulation fails to bring about a change in the morphology of mitochondria in the tropochrome cells. Further, stimulation of the chorda tympani results in a change from the filamentous form of mitochondria in the resting cell to the globoid form, in both types of cells. This lack of change in the mitochondria of the tropochrome cells following sympathetic stimulation is difficult to explain. It might easily be due to the fact that stimulation was never continued longer than one hour. Stormont continued sympathetic stimulation for a period of six hours. Further, the type of current used may have been unsuitable for stimulation of the sympathetic, although a similar current resulted in signs of physiological activity when applied to the chorda tympani.

SUMMARY

The chorda tympani and sympathetic nerves to the submaxillary gland of the rabbit were isolated and stimulated for varying lengths of time (five minutes to one hour). The opposite gland was used as a control.

Changes in morphology of mitochondria of the tropochrome and homeochrome cells occurred following stimulation of the chorda tympani. These changes consisted of a transition from filaments to short thick rods and finally to globoid mitochondria. The maximum change occurred at twenty minutes, in which case the majority of the mitochondria were globoid in form.

No definite change in mitochondria followed stimulation of the sympathetic nerve supply. Stimulation of the sympathetic failed to dislodge the serozymogen granules of the homeochrome cells. Stimulation of the chorda tympani dislodged the serozymogen granules and resulted in a morphological change in the mitochondria of the tropochrome and homeochrome cells. Stimulation caused no change in the mitochondria of the ducts.

The mitochondria showed no increase or decrease in numbers following stimulation. There was no indication of their being used up in the elaboration of secretion.

A brief description of the mitochondria in the resting gland is reported.

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PLATES

DESCRIPTION OF PLATES

Technical remarks. All the figures are untouched photomicrographs made with Wratten 'M' plates and Velox paper. A B. & L. illuminating unit, 6-volt spiral-filament lamp with green and yellow ground-glass screens were used in all cases. In every case a B. & L. aplanatic condenser, and a B. & L. fluorite oil immersion, numerical aperture of 1.32 mm., with a B. & L. 10× Huygenian ocular were used. The magnification for all illustrations is × 1400.

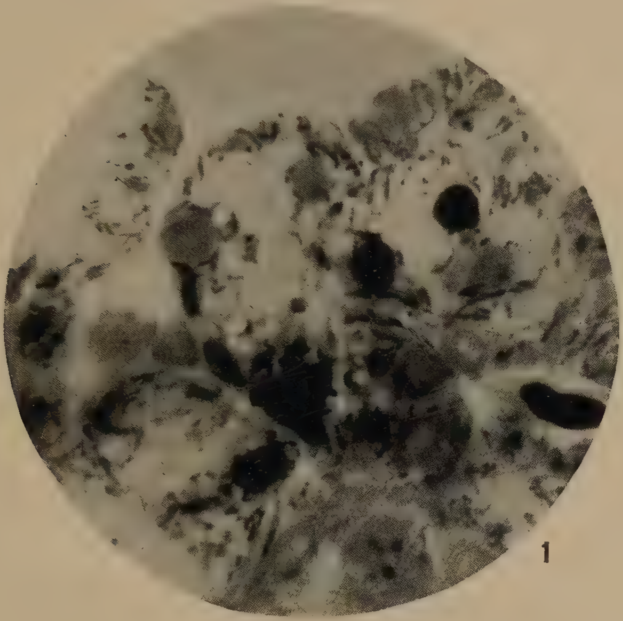
PLATE 1

EXPLANATION OF FIGURES

1 Tropochrome acini of a normal control from the left submaxillary gland of the rabbit, fixed and stained by the Cowdry modification of the Regaud-Altmann method for demonstration of mitochondria. This method was used in all cases. Note that the long irregular filamentous type of mitochondria predominate and appear slightly more numerous near the basement membrane. An occasional globoid form may be seen. A few filaments show a bulb-like swelling at one terminal.

2 Tropochrome acini in the submaxillary gland of a rabbit, showing effects of twenty-minute stimulation of the chorda tympani nerve. Note particularly the globoid type of mitochondria. No filaments are noticed. Short thick rods are present. In places the globoid forms are arranged in chains, suggesting the possibility of their origin from a single filament. In the lower left-hand corner of the field a small portion of a large collecting duct with its content of thick rod-like mitochondria can be seen.

PLATE 1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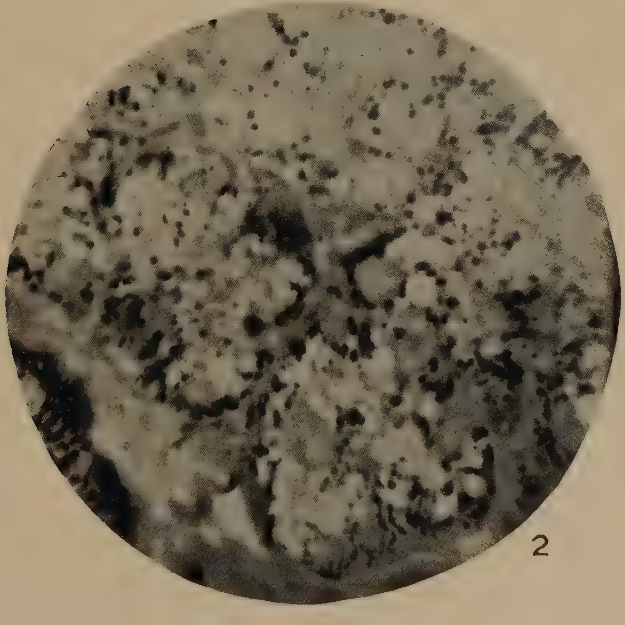
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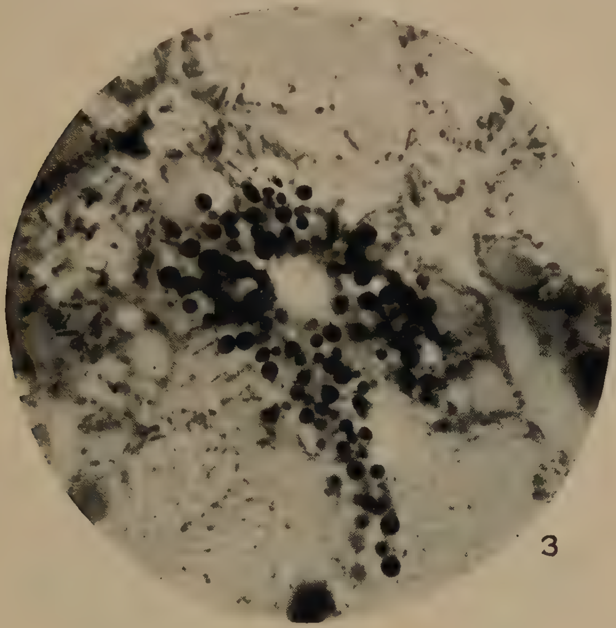
PLATE 2

EXPLANATION OF FIGURES

3 A normal control homeochrome acinus from the submaxillary gland of a rabbit. The acinus is filled with serozymogen granules. The mitochondria are present in the form of filaments between the serozymogen granules. They are most numerous near the basement membrane and around the nuclei.

4 An exhausted homeochrome acinus, following twenty minutes' stimulation of the chorda tympani nerve. Although small rod-like forms can be seen, the globoid type predominate and they are very small. A single serozymogen granule is present in upper portion of the acinus. There is no apparent increase or decrease of the mitochondrial content of the acinus.



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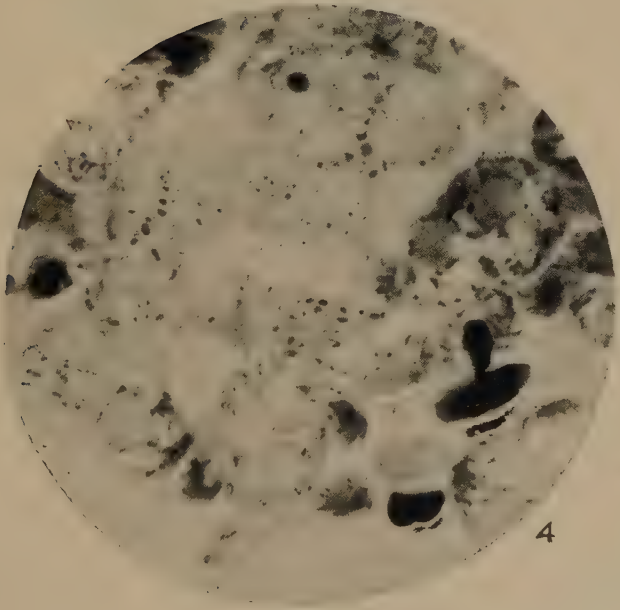

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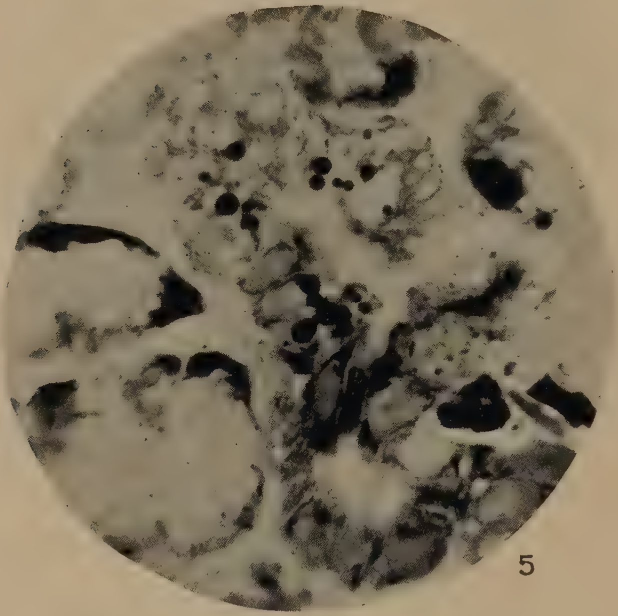


5 A terminal intercalary duct in the submaxillary gland of the rabbit. The gland has been subjected to combined stimulation of the chorda tympani and the sympathetic trunk for fifteen minutes. The non-secretory duct cells are present in the lower portion of the picture. Note that the mitochondria are collected around the nuclei. The individual details cannot be discerned. The distal portion of the cytoplasm of the cell is free. A few serozymogen granules are present. Note the filamentous mitochondria near the upper portion of picture in the homeochrome cells of the acinus.

THE EFFECT OF NERVE STIMULATION AND NERVE DEGENERATION ON THE MITOCHONDRIA AND HISTOLOGY OF THE THYROID GLAND

I. H. SMITH AND H. C. MOLOY

Department of Pathology and Bacteriology, University of Western Ontario Medical School, London, Canada

FOUR FIGURES

Histological, physiological, and chemical evidence has been produced in favor of, and in opposition to, nerve control of thyroid secretion. The difficult problem in establishing final proof of nerve regulation has been in deciding on a proper index to represent gland activity. Recently, Moloy and Smith ('30) have shown that in the submaxillary gland of the rabbit definite changes occur in the morphology of the mitochondria after activation of the gland by nerve stimulation. Cramer and Ludford ('26), as well as others, have observed that the mitochondrial constituents of the thyroid vary in a precise manner with thyroid activity, but as yet no attempts have been made to produce such mitochondrial alterations in the thyroid by stimulation or section of thyroid nerves—this is the object of our series of experiments. A brief review is given below of the histological, physiological, and chemical studies which have advanced evidence both in favor of and against nerve control of thyroid secretion.

HISTOLOGICAL STUDIES

Degeneration of the gland is reported to have followed division of the laryngeal nerves (Katzenstein, '97) or of the cervical sympathetic trunk (Missiroli, '09). Stimulation of the cervical sympathetic from time to time over a period of one to two and one-half months has produced changes indicative of increased activity, while changes indicative of decreased activity have been reported following excision of the cervical sympathetic (Reinhard, '23). This work has not been confirmed by later workers (Schiff and Heinrick, '24). No histological changes have been observed after section or stimulation of either the cervical sympathetic trunk or the vagus and its branches (Crawford and Hartley, '25). Stimulation of the superior cervical ganglion, or injecting B. bronchisepticus into it, has been reported as producing either thyroid hyperactivity or exhaustion, according to the duration of the stimulus (Wilson, '18). The same investigator ('16) has observed degenerative lesions in the cervical ganglia in cases of exophthalmic goiter. No histological difference could be detected between a graft of thyroid in which nerve control was absent and the part of the gland which remained after partial thyroidectomy (Manley and Marine, '16).

PHYSIOLOGICAL STUDIES

Marine ('23) found no change in the functional capacity of the gland after the nerves running in the adventitial coats of the vessels had been sectioned and the gland encased in paraffin. Stimulation of the cervical sympathetic has been stated to cause an increased response to adrenalin after a latent period of one hour. Previous thyroidectomy prevented this action, but adrenalectomy did not alter the response (Levy, '16). Cannon and Smith ('22) described an increase in the rate of the denervated heart after massage of the thyroid or stimulation of the cervical sympathetic. In both instances they consider the reaction to be caused by a discharge of thyroid secretion. Cannon, Binger, and Fitz ('14) produced symptoms resembling those of exophthalmic goiter by performing a phrenicocervical sympathetic anastomosis; but Marine and his coworkers could not confirm their work.

CHEMICAL STUDIES

Stimulation of the vagosympathetic trunk, or of the superior thyroid vessels with their accompanying nerves, has been reported to cause a reduction of the iodine content of the gland (Rahe, Rogers, Fawcett, and Beebe, '14; Watts, '15). Van Dyke ('21) fails to agree with these observations. Removal of the inferior cervical ganglion has been reported to cause a loss of weight and reduction of the iodine content, while section of the vagus was without effect (Wiener, '09).

FACTORS ALTERING THE MORPHOLOGY OF MITOCHONDRIA IN THE THYROID

Cramer and Ludford ('26) induced intense activity in the thyroid of the rat by exposing the animal to an environment of low temperature or by the subcutaneous injection of tetrahydronaphthalamine. They decreased the activity of the gland by exposing the animal to an environment of high temperature or by thyroid feeding. With increasing activity the mitochondria enlarged and became filamentous; with diminished activity a reduction in the number of mitochondria occurred which was accompanied by granulation.

Nicholson ('24) also has observed that by thyroid feeding mitochondria assume granular forms. By partial thyroidectomy he has produced an increase in the number of mitochondria.

Seecof ('25) has shown in hyperplasia of the thyroid produced by fat feeding or by partial thyroidectomy that "during the developmental stages of active hyperplasia mitochondria increase in number, become filamentous and concentrate at the lumen pole of the cell. During involution as obtained by iodine administration the mitochondria decrease in number, become shorter and more scattered." He arrived at the conclusion that the mitochondrial content is dependent on the iodine-percentage content of the cell, just as Marine has shown that histological changes are dependent on the same factor.

The above brief review of the literature has brought out the following points: 1) there is no final agreement as to the effect of nerve stimulation or section on the activity of the thyroid gland; 2) mitochondria of the thyroid respond to certain factors which increase or decrease the activity of the gland; 3) mitochondria of the thyroid respond to factors (artificial hyperplasia or involution) which alter the percentage of the iodine content of the gland; 4) mitochondria of the submaxillary gland show definite morphological changes after nerve stimulation.

Taking into consideration these four different facts, one can merely theorize as to what effect either nerve stimulation or section would have on the mitochondrial content of the thyroid. The experiments recorded below were performed with the object of throwing some light on this theoretical question.

MATERIAL AND METHODS

Twenty-five healthy adult rabbits were used in this series of experiments; of these, only two were females. The diet used throughout was composed of cabbage, turnips, oats, and water.

In the nerve-stimulation experiments the isthmus of the thyroid was exposed and ligated with plain catgut, under ether or urethane anaesthesia. The nerves to be stimulated were then isolated, placed on platinum electrodes, kept warm and moist, and stimulated for short intervals with faradic currents of a strength just perceptible on the tip of the tongue. Artificial respiration occasionally was employed; if so, injury to the inferior thyroid vessels and recurrent laryngeal nerves was guarded against. At the end of periods ranging from fifteen to sixty minutes the lobes were removed.

In the nerve-degeneration experiments ether was always used as the anaesthetic. Aseptic technic was rigidly employed, alcohol being used exclusively as the antiseptic, iodine being strictly avoided. As in the stimulation experiments, the isthmus of the gland was exposed and ligated. The nerves were then isolated and resected a distance of approximately 1 cm. The subcutaneous tissue and skin were closed with interrupted linen sutures. Sepsis was never encountered. At the end of intervals varying from fourteen hours to eleven days the animals were again etherized and both lobes were removed.

The tissues to be examined for mitochondria were cut into blocks which never exceeded a diameter of 4 mm. Fixation in Regaud's fluid, subsequent mordanting, clearing, and embedding, as well as staining with acid anilin fuchsin and methyl green, were all carried out according to the method given by Cowdry ('18). The sections stained by this technic were cut between 2 and 4 μ in thickness.

The tissues to be stained by hematoxylin and eosin were fixed in 10 per cent formaldehyde. Sections were cut at 4 μ.

OBSERVATIONS

Normal rabbit thyroid histology

The control lobe in each experiment is used to represent normal tissue. Briefly stated, the individual picture is extremely variable, ranging from one composed of much dilated acini filled with colloid to one of solid acini containing no colloid. In general, the height of the epithelial cell varies inversely as the size of the acinous lumen; i.e., high cuboidal cells line non-dilated acini, while low cuboidal cells line much dilated acini. Interfollicular cells are constantly present; vacuolation of colloid is observed in many instances.

Mitochondria of the normal rabbit's thyroid

As in other gland cells, the mitochondria of the rabbit's thyroid are seen as granules, rods, and filaments. The predominance of one or other form is dependent largely on the height of the individual cell. The number per individual cell is decidedly variable. These points have been covered in detail by Seecof ('27), who has given us a cytological classification of thyroid cells on a quantitative mitochondrial basis, namely: chief, mitochondria-rich, and mitochondria-poor cells.

The *chief cells* (figs. 1, 2, 3, and 4), as the name implies, comprise the main cells of the acinus. All forms of mitochondria may be present in a single cell, but the filamentous types tend to predominate in the high cuboidal, while granular and rod-like types are associated most frequently with the low cuboidal cell. The tendency of the mitochondria, especially those of granular form, to accumulate at the cell apices, i.e., about the periphery of the lumina, is quite a notable characteristic (fig. 4). This feature is most pronounced in the more dilated alveoli.

Mitochondria-rich cells (figs. 1, 2, and 3) are characterized by their high mitochondrial content. The individual mitochondrial forms vary, as in the chief cells, with the height of the individual cell. The filaments are shorter and not so wavy as in the chief cell. The mitochondria are thicker in cross-section and are packed very closely together. In some instances the mitochondria have fused into a solid red-staining mass. The density of these cells contrasts decidedly with the chief cells.

Mitochondria-poor cells (figs. 1 and 3) are invariably round or oval in outline. When stained by Cowdry's method, they

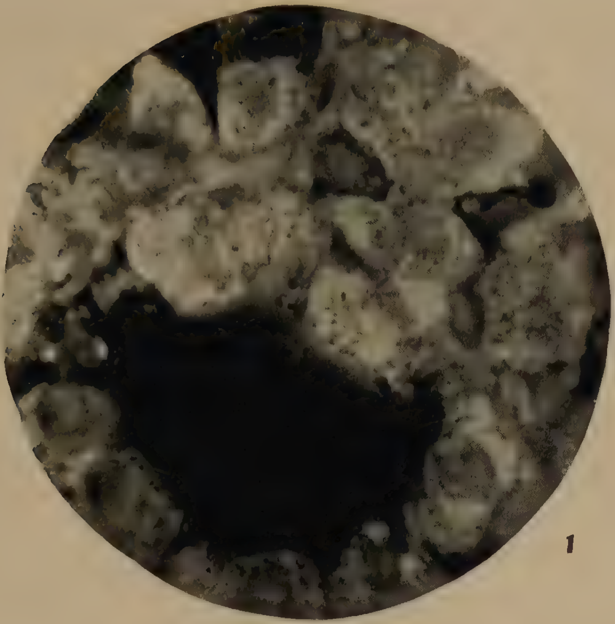
EXPLANATION OF FIGURES

Each illustration is an unretouched photomicrograph of the thyroid gland (rabbit); Regaud's fixative; sections 3 μ in thickness stained for mitochondria by the acid-anilin-fuchsin technic.

Material. All figures were produced on Wratten M plates and Velox paper. The source of light was from a Bausch & Lomb 6-volt spiral-filament illuminating unit, filtered through green and yellow glass screens. In every instance the following were used: a B. & L. 10× Huygenian ocular; a B. & L. fluorite oil-immersion 1.9-mm. lens with a numerical aperture of 1.32; a B. & L. aplanatic condenser immersed in oil. The magnification for all illustrations is × 1400.

Fig. 1 Taken from the left thyroid lobe in a nerve-degeneration experiment in which a unilateral section of the right cervical sympathetic was performed. Acinus is composed of high cuboidal epithelial cells. Filamentous mitochondria predominate. Two mitochondria-rich cells are prominent on the left; these are black, as contrasted with the two mitochondria-poor cells above. The mitochondria in these large oval mitochondria-poor cells are filamentous, as in the chief cells.

Fig. 2 From the lobe on the experimental side (right); same experiment as in figure 1. Lower type of cell than in figure 1. Mitochondria are granular and rod-like. Very few filamentous forms are present. Two characteristic mitochondria-rich cells are present on the left in which the individual mitochondrial forms cannot be clearly identified. Vacuolation of colloid.



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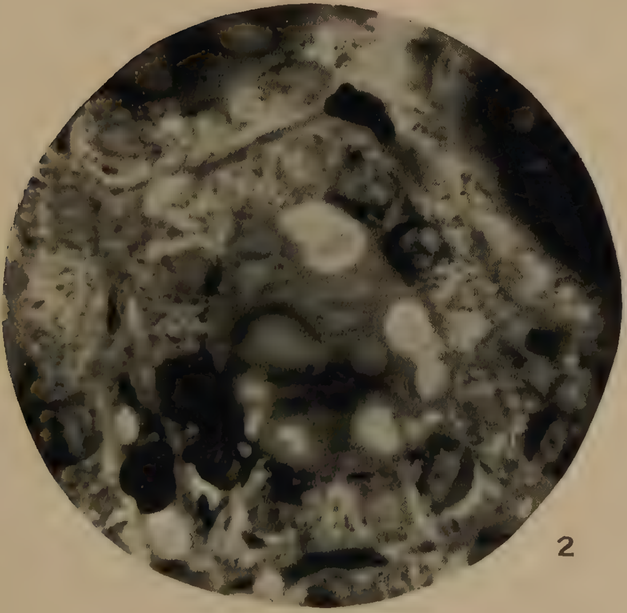

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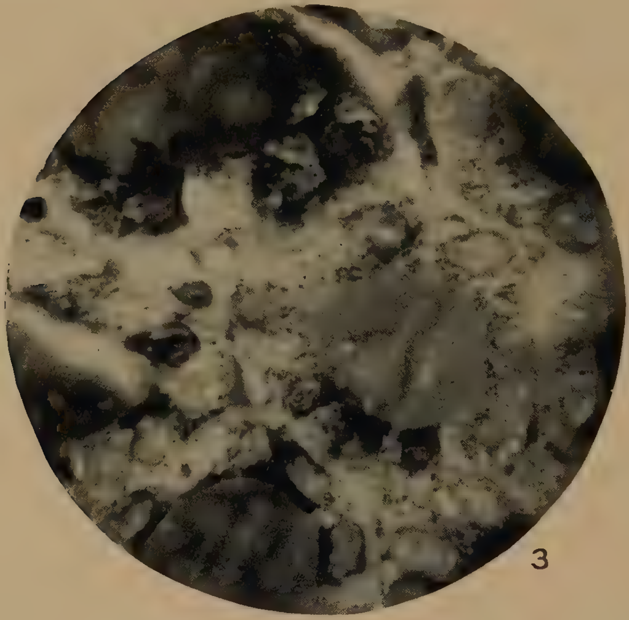
Figures 1 and 2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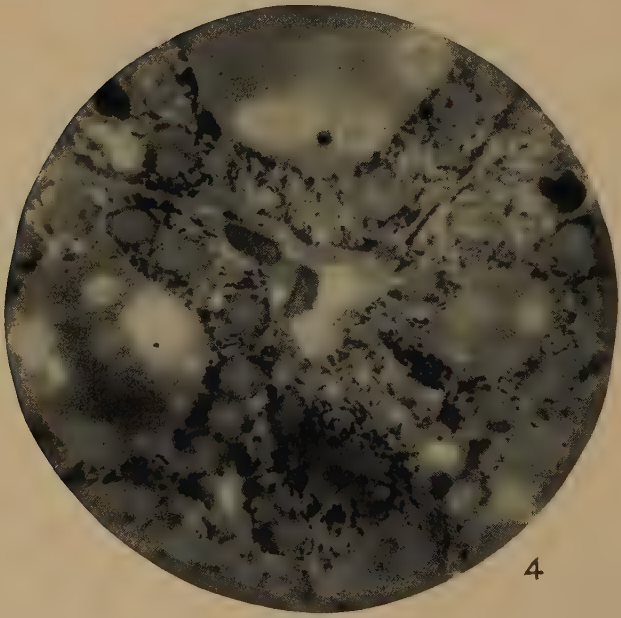
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Figures 3 and 4

have a very pale green cytoplasm and contain relatively fewer mitochondria than the other two types. The mitochondria tend to be granular and rod-like rather than filamentous; filaments, however, in our experience are quite common. All forms tend to be more minute than in the chief cell, but not necessarily so. They are distributed uniformly throughout the cytoplasm or tend to encircle the nucleus. Not only are these cells found in the acini, but, like the chief and mitochondria-rich cells, they are occasionally seen between the follicles.

Invariably, mitochondria-rich cells are most numerous in sections made up of large dilated acini filled with colloid and lined with low cuboidal epithelium. In alveoli with still lower epithelium they are even more numerous. They are relatively few in smaller acini with a higher cuboidal epithelium. In the glands showing alveoli with high cuboidal cells and with small lumina having no colloid, mitochondria-rich cells are a rarity, but mitochondria-poor cells are numerous. It was also noticed that when mitochondria-rich cells lie beside mitochondria-poor cells in acini moderately dilated, they appear in height to be approximately half that of the mitochondria-poor cells (fig. 1).

The effect of nerve stimulation

The nerves on the right side were stimulated in each instance. The experiments performed were as follows: *a*) Section of the cervical sympathetic trunk as low in the neck as possible, with stimulation over periods varying from

Fig. 3 From the same preparation as figure 2. The cells are of the average height. Several mitochondria-rich cells are present. The mitochondrial forms of the mitochondria-rich cells are quite distinct and appear thicker in cross-section than those of the chief cells. Granules, rods, and fine filaments are all present in the chief cells. A mitochondria-poor cell is distinctly prominent in the upper part of the picture between two mitochondria-rich cells.

Fig. 4 From stimulated lobe (sympathetic stimulation sixty minutes). Composed entirely of chief cells in which granules and short rods predominate. Tendency to accumulation in the cell apices. Mitochondria are embedded in the colloid (acinus lower part of field).

fifteen to sixty minutes (eleven experiments). *b*) Section of the vagus above the superior laryngeal, with stimulation for fifteen to thirty minutes (five experiments). *c*) Section of the cervical sympathetic trunk, isolation of the superior and recurrent laryngeal nerves, stimulation of all three nerves over a one-hour period (one experiment). *d*) Stimulation alternately for fifteen minutes of the recurrent laryngeal nerve and the vessels and nerve fibers entering the superior pole (one experiment). *e*) Stimulation of the superior and recurrent laryngeal nerves for fifteen minutes each (one experiment). *f*) To ensure that no fibers entering the gland were being missed, the vessels and nerves entering both upper and lower poles were stimulated for forty minutes (one experiment). This involves a total of twenty nerve-stimulation experiments.

In examining the preparations stained for mitochondria we focused our attention principally on, 1) alterations that might occur in either the quantity, the morphology, or the distribution of the mitochondria, in the chief, mitochondria-rich, and mitochondria-poor cells; 2) any increase or decrease in the number of the two latter types of cells.

Considering these possible variations, *no change was observed in the mitochondrial elements of the stimulated lobe as compared with the control.*

On examination of the hematoxylin-eosin preparations, it was found that *invariably the histological picture of the stimulated lobe was similar to the control.* This observation confirms the findings reported by Crawford and Hartley ('25).

The effect of nerve section

In each instance the nerves sectioned were on the right side. A total of five nerve-degeneration experiments were performed as follows: *a*) Section of the vagus above the origin of the superior laryngeal, and section of the cervical sympathetic trunk low in the neck (three experiments); two of these animals were killed at the end of seven days and the third at the end of twelve days. *b*) Section of the cervical sympathetic trunk alone, low in the neck (one experiment); the animal was killed after eleven days. *c*) Section of the cervical sympathetic trunk and the recurrent laryngeal (one experiment). The lobes were removed after seven days.

No alteration in the mitochondrial content of the right (degenerated) side was observed as compared with the lobe on the left.

The histological picture of the thyroid lobe on the right (experimented) side was in each instance the same as the lobe on the left.

DISCUSSION AND CONCLUSIONS

By using mitochondria to represent cell activity we do not assume that mitochondria are precursors of colloid, for how such structures as mitochondria, made up chemically as they are of a phospholipin combined with a protein (Bullard, '16; Regaud, '08), can form such diverse products as zymogen granules, colloid, etc., is not clear to us. Grynfeltt ('12) has suggested that a direct relationship exists between the formation of thyroid colloid and mitochondria. Key ('25) holds no such views, but suggests rather, as do other workers, including Cowdry ('18) and the Lewises ('15), that these cytoplasmic elements wherever they are found play some unknown rôle in cell metabolism and cell respiration. From our observations on the submaxillary gland of the rabbit we also favor the view held by these latter workers. The fact that they show an alteration in the activated gland, however, presupposes that they are not inert, but play an important, active, yet not specific, rôle in cell activity. It is from our observations on the alteration in the morphology of mitochondria in the activated submaxillary gland of the rabbit that we feel justified in using this cytological index to represent gland activity. That mitochondria are constantly changing in size, shape, and position in tissue cultures has been shown by the Lewises ('15); that they assume granular forms in the resting thyroid and filamentous forms in the active gland is, however, worthy of consideration. We have observed the exact opposite to hold true in the submaxillary of the rabbit. If, then, so constant a mitochondrial variation is present after such activity and inactivity of the thyroid gland, should we not expect some similar change to occur after activating and inactivating the gland, as previous workers claim to have done by stimulation or section of thyroid nerves?

Study of mitochondria of the epithelial cells reveals no change, either morphologically, quantitatively, or topographically, following stimulation or section of the cervical sympathetic trunk or of the vagus and its branches. Such an observation, in view of our positive findings in the submaxillary, leads us to believe that further evidence against nerve control of thyroid secretion has been produced. It is true that, in activity, the physical properties of the cytoplasm of the submaxillary cell (including surface tension) may be altered to a much greater degree than that of the thyroid cells. This may account for the remarkable change in the submaxillary and the absence of change in the thyroid. Again, it is possible that faradic stimuli sufficient to give change in an external secreting gland may fail to produce activity in the thyroid. The ideal way to overcome this drawback would be gradation of stimuli with different apparatus, but such an undertaking was not within our power.

We agree with Seecof in his observations on the morphology of the mitochondrial constituents of the epithelial cells which varies with the height of the individual cell. Although filamentous, rod-like, and granular forms may be found in a single cell, nevertheless granules and rods predominate in low cuboidal cells, while filaments are more common in the high cuboidal cells. Furthermore, the length of the filaments varies directly as the height of the cell within which they are contained.

The so-called 'mitochondria-rich,' 'mitochondria-poor,' and 'chief cells' of Seecof are readily identified in the thyroid gland of rabbits.

Neither unilateral stimulation nor unilateral section of the cervical sympathetic or of the vagus and its branches produces any alteration (detectable by the methods which we employed) in the histological structure of the lobe on the experimental side as compared with the control lobe.

It is a pleasure to acknowledge the many helpful suggestions offered by Dr. H. H. Bullard during the progress of this work on mitochondria in the submaxillary and thyroid glands. The authors also wish to express their thanks to Dr. E. V. Cowdry for the helpful interest he has taken in these papers, and to Mr. C. E. Brine for the microphotography hints he has so willingly tendered.

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